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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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REPRESENTATION OF THE INTERNAL ORGANS IN THE CEREBRAL AND CEREBELLAR CORTICES OF CATS AND DOGS

PART V. Individual Differences in the Specific Cortical Projection Areas

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
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When studying the specific projection areas of various visceral nerves by making electrical recordings, we found considerable individual variations in their disposition on the cortical surface [2].

We have previously described [2, 3] the specific projection areas of the pelvic nerves on the cortical surface in cats and dogs, and we found that these areas occupy definite positions in the cortex. They are found in the same position in most animals and this may therefore be considered to be the "basic variant" position. However, in some of the experiments we have encountered considerable deviations.

Dogs show greater variations than cats, though the arrangement of gyri and fissures is more variable in the latter.

Thus, for instance, I. Filimonov [1], in considering only the coronary and sypreasylvian fissures, distinguishes ten variations in cats and dogs. In both animals, the commonest type is shown in the atlas published by the State Brain Institute [1]. This atlas also gives the "basic variant" of the area of representation of the nerves we have studied [2, 3].

It appears that no attention has been paid to the considerable and very important variations in localization, as we have found no published information on this point.

The results described below give some idea of the possible individual differences in the site of the specific projection areas and on the nature of the cortical structure associated with these variations.

RESULTS

Cats. Most frequently, the first area of representation of the pelvic nerves lies on the posterior cruciate gyrus, enclosing a small portion of the adjoining anterior cruciate gyrus. Within this zone, the focus of maximal acitivity (FMA, i.e., the cortical area from which primary electrical potentials having the smallest latent period and greatest amplitude may be recorded) as a rule lies on the posterior cruciate gyrus where it can be seen to spread along the cruciate fissure.

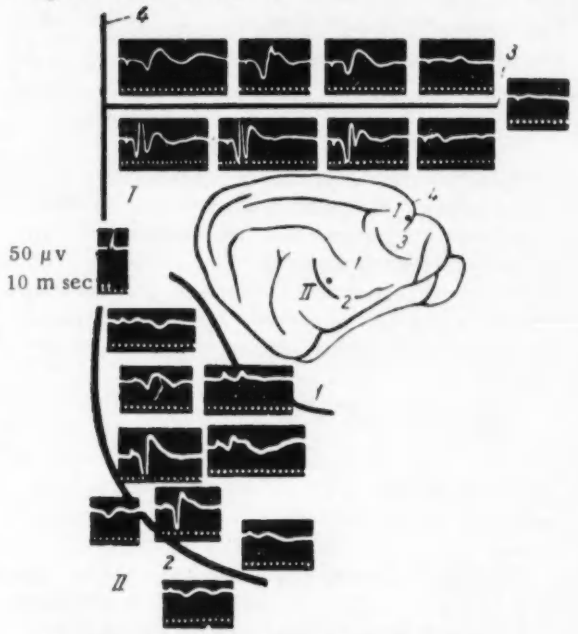

Fig 1. Disposition of the primary (I) and secondary (II) areas of representation of the pelvic nerves. The black circles indicate foci of maximal activity (FMA). The records of the potentials illustrate the responses from the first and second representational areas. 1) Anterior suprasylvian gyrus; 2) anterior ectosylvian gyrus; 3) cruciate gyrus; 4) longitudinal fissure. Chloralose anesthesia.

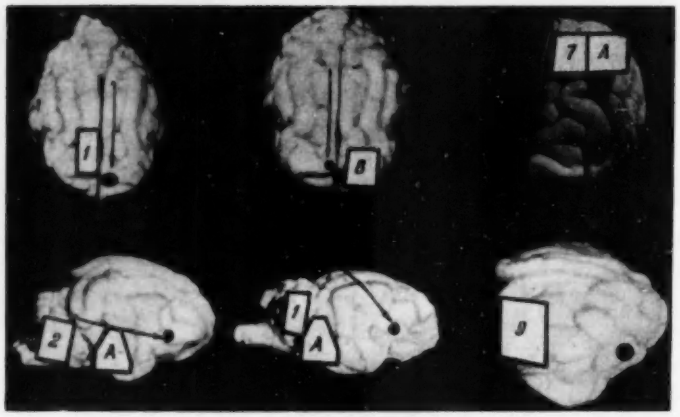

Fig. 2. Variations in the representation of the first (preparations 1, 8, and 7A) and the second (preparations 2A, 1A, and 9) contralateral cortical areas of representation of the pelvic nerves.

Figure 1 shows the results on an experiment in which one of the commonest positions of the FMA of the pelvic nerves occurred in the primary projection area. As can be seen, it lay at a short distance from the median portion of the cruciate fissure. Figure 2 shows some variants of this position which we observed within the area (preparations 1, 8, and 7A).

Thus, in preparation 1, the area lies on both sides of the cruciate gyrus and extends somewhat further than usual on to the anterior cruciate gyrus.

In preparation 8, the area is displaced onto the caudal portion of the posterior cruciate gyrus, while in preparation 7A it has been moved forward to the anteriomedial portion of the lateral gyrus.

There are individual differences in localization not only in the first field of common somatic sensitivity, but similar variations may also be observed in the second field.

Figure 1 (II) shows the results of an experiment which demonstrates one of the commonest positions of the FMA of the pelvic nerves encountered in the second cortical projection area. It lies at the front of the ectosylvian gyrus near the anterior portion of the anterior ectosylvian fissure.

Preparation 2A (see Fig. 2) shows the principal position of the second cortical representation area of the pelvic nerve. The Figure also shows preparations 1A and 9, which are two examples of an atypical disposition of the area.

The greatest displacement of the area is shown in preparation 9, and here the characteristic cortical structure should be noted, and it can be seen that it differs considerably from that of preparations 2A and 1A.

Dogs. In spite of the limited number of experimental animals [16], individual differences in localization of the area were found (Fig. 3).

Most frequently, the first representational area of the pelvic nerves in dogs lies over the presplenial gyrus, 1.5-2 mm more caudal and more medial than is the case in brain No. 1 of Fig. 3. In another experiment, the areas were found on the posterior cruciate gyrus in the middle portion of the anterior cruciate fissure (see Fig. 3, brain No. 2).

The most typical position of the second area (see Fig. 3) is shown in brain 2A, and by comparing this with preparation 1A it can be seen that there is a considerable displacement of the projection area.

In 1941, Marshall, Woolsey, and Bard [6] showed that in the first musculocutaneous sensory area in the cat, two foci of representation of the forelimb could be found. In their experiments, a weak tactile stimulus was applied to the hairs of the dorsal circuit of the front paw, which, besides causing a response from the second contralateral area, also elicited primary responses in two regions of the first contralateral area. They showed that each portion from which maximal primary responses could be obtained was surrounded by a characteristic border zone which gave submaximal responses. The same phenomenon was found in the ape brain, where stimulation of any part of the body always produced primary responses in two or more regions of the area representating that part of the body.

Adrian [4] has questioned these results, but Marschall [7] subsequently confirmed them in experiments on cats.

Amassian [5] has shown that the splanchnic nerve may have multiple cortical representations. In some experiments he made observations which supported this hypothesis, but reached no definite conclusions.

We found that in some of the animals, stimulation of the pelvic nerves, both in cats and in dogs, caused activity in two separate foci. This occurred in both the first and

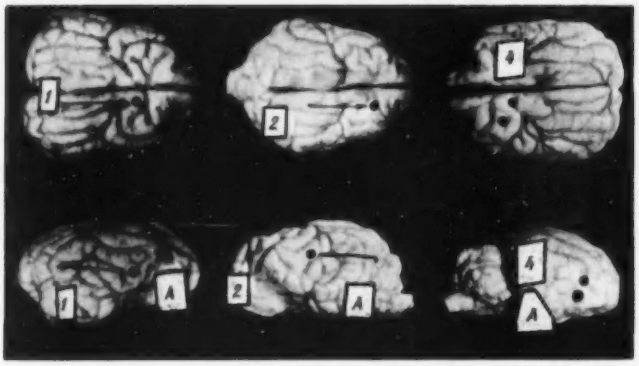

Fig. 3. Variations in the localization of the first (preparations 1 and 2) and second (preparations 1A and 2A) contralateral representational areas of the pelvic nerves in dogs. Preparations 4 (dog brain) and 4A (cat brain) show a case of double representation of the pelvic nerves in the first and second contralateral cortical areas.

second representational areas. Regions in which large electrical responses occurred might be close together, but were usually separated by some distance.

Figure 3 (brain No. 4) shows two foci in the first cortical representational area of the dog brain which responded to stimulation of the pelvic nerve.

Brain No. 4A shows a similar instance which occurred in one of the experiments on the cat. Stimulation of the pelvic nerve caused a primary response to develop in two regions of the second area, while in the first area only one sensitive region was found which was very small (smaller than usual).

It must be noted that this kind of multiple representation is most frequently encountered under light chloralose anesthesia.

These multiple foci indicate not only differences in localization, but differences in individual organization (in the broadest sense of the word) of cortical representation.

As has been shown, individual differences in cortical representation of the pelvic nerves may result on the one hand, from a displacement in the representational area of the limbs. On the other hand, it is quite probable that this displacement is associated with a shift of the whole musculocutaneous representational area. This phenomenon was observed by us in the cat whose brain is shown as No. 9 in Fig. 2. In this experiment, simultaneous stimulation of the sciatic nerve showed that the area in which the primary responses occurred was equally displaced downwards and surrounded the area of the pelvic nerve, being stretched in the dorsocaudal direction.

It must be emphasized, in these cases, when it is required to give an accurate description of the localization of one or another cortical representational area, it is essential to make a careful choice of experimental animal with respect to weight, age, and breed in order that results obtained from animals with a common cortical structure may be obtained.

If it is required to make a preparation with electrodes chronically implanted into any particular sensory area, in order to avoid mistakes being made it is important first to accurately determine the topography of the areas on the surface of the cortex.

Such a determination may be made under sterile conditions at operation immediately before implanting the electrodes.

The same precaution applies also when any particular area is to be damaged or removed.

SUMMARY

It was shown that in different animals of the same species there were distinct differences in the positions of the representational areas of both visceral and somatic nerves. The differences were associated with the individual development and structure of the cerebral cortex. The results should be taken into consideration when performing various operations on the cortex such as implanting electrodes or extirpating certain areas.

LITERATURE CITED

1. S. A. Sarkisov and I. N. Filimonov (Editors), Atlas of the Brain of Man and Animals [in Russian] (Moscow, 1937).
2. K. M. Kullanda, Byull. Éksp. Biol. i Med. 43, 3 (1957).*
3. K. M. Kullanda, Representation of Some Internal Organs in the Cerebral and Cerebellar Cortices of the Cat and Dog (Candidate's dissertation) [in Russian] (Moscow, 1958).
4. E. D. Adrian, J. Physiol. 100, 159 (1941).
5. V. E. Amassian, J. Neurophysiol. 14, 435 (1951).
6. W. H. Marshall, C. N. Woolsey, and P. Bard, J. Neurol. (1941).
7. W. H. Marshall, Fed. Proc. 8, 107 (1949).

* Original Russian pagination. See C. B. Translation.

EFFECT ON THE HEART RATE OF TREATING MUSCLES WITH HYPO-, ISO-, AND HYPERTONIC SOLUTIONS

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Reflex visceral and skeletal muscle changes may be induced by perfusing [1, 7, 13], injecting [3, 14], or applying various chemical substances to the surface of the muscles of limbs which retain only a nervous connection with the rest of the body. Chemical stimulation of proprioceptors must therefore take place, and this view is confirmed by recording potentials from the sensory nerve supply to the muscle while the latter is treated with salts and other substances [4, 16].

We have studied the effect on muscle of distilled water, isotonic saline (0.65 % NaCl), and hypertonic saline (1.3 and 1.95 % NaCl); Ringer's solution was used for the control. Reflex changes in the activity of the heart and skeletal muscle were used as indicators of proprioceptor stimulation.

METHOD

The experiments were carried out on 20 Spring and 20 Autumn male frogs (Rana temporaria).

Before the experiment, a cut was made through the skull bone to divide the brain between the medulla and the optic lobes. The heart was exposed and so were the muscles of the anteromedial surface of both thighs, or all the muscles of both hindlimbs.

The experiment was begun 45-60 min after dividing the brain, after recovery from spinal shock. The heart contractions were recorded on a kymograph. Every 2-4 min one of the solutions was applied on a piece of filter paper 6 · 8 mm to the m.cruralis, or, less frequently, to the gastrocnemius muscle. The period of stimulation was varied from a few seconds to 1 ½-3 min. The electrocardiogram was recorded both during the application of the stimulus and for a few minutes after it had been washed out with Ringer's solution.

RESULTS

As was to be expected, the application of Ringer's solution to muscle had no effect on the heart rate.

Sodium, potassium, and calcium ions must be present in a certain proportion for normal functioning of the neuromuscular apparatus to take place. It would therefore be expected that stimulating the muscle with different concentrations of NaCl would bring about proprioceptor changes.

Physiological saline (0.65 % NaCl) caused no change in the heartbeat or in the functioning of the other organs, the probable reason being that the diffusion of salt, which may change the ionic composition and properties of muscle, takes place quite slowly.

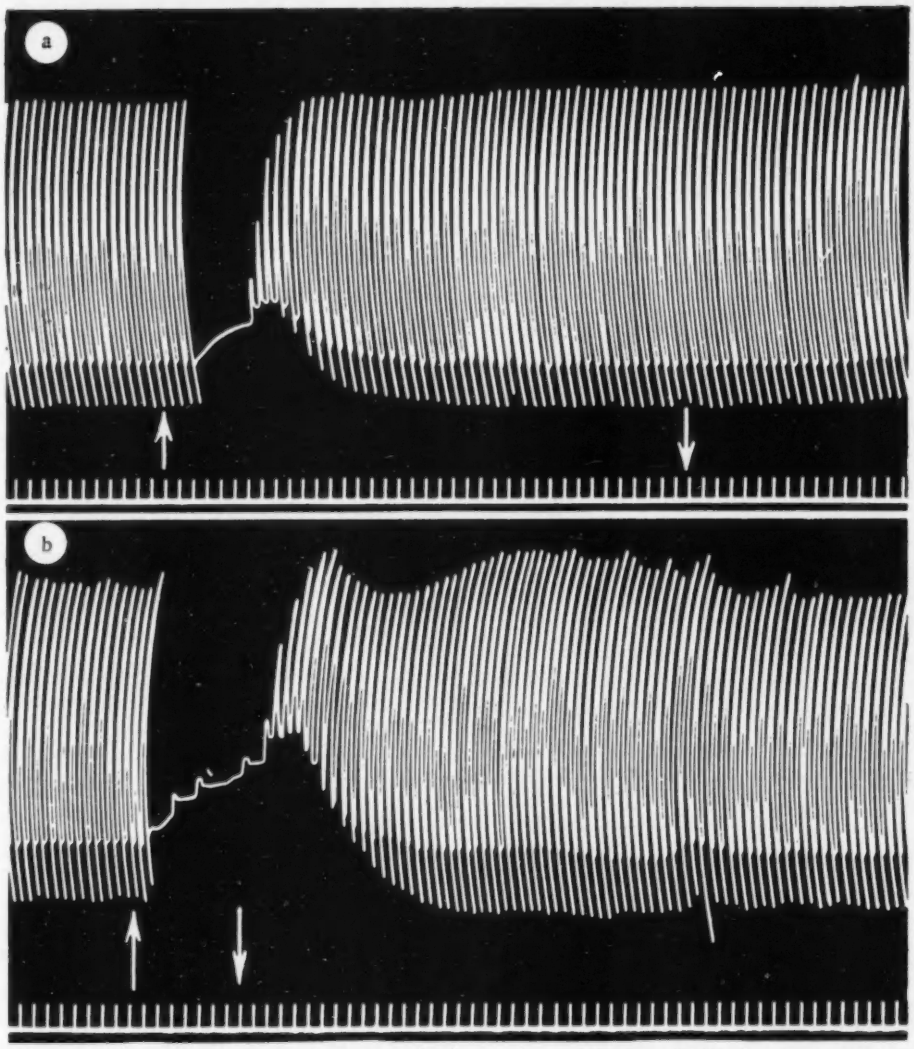
When the muscles were stimulated by a 1.3 % sodium chloride solution, in seven out of the 14 tests, the heartbeat was affected.

When a 1.95 % solution of NaCl was applied to the surface or used to irrigate the muscle, the heartbeat changed in 42 % and 84 % of the cases respectively.

When the thigh muscles were irrigated with 1.95 % NaCl, the solution might reach the inner skin surface and so stimulate cutaneous receptors. For this reason, experiments were carried out in which the skin of one limb and the muscles of the opposite limb from which all skin had been removed were irrigated alternately. Under these conditions, cutaneous stimulation brought about the same cardiac changes and the same motor responses as when the thigh muscles were irrigated. However, the latent period of the response induced by irrigating the muscles had an average value which did not exceed 13 sec whereas when the skin was irrigated the latent period had an average value of 90 sec.

When a "hypotonic solution" consisting of twice-distilled water was used, cardiac and skeletal-muscle changes occurred which resembled those induced by hypertonic solutions (see Figure).

Cardiac changes occurred in 127 of the 166 tests with water. In 118 cases, the amplitude of the contractions was reduced and then returned to the original value either immediately, or after a temporary increase or fluctuation in amplitude. Whether the water was applied on filter paper or by irrigation, the amplitude change was about 26-36%; the change lasted for 81-100 sec. In 89 out of the 166 tests the heart rate was slowed on average by 30 % for 45-46 sec. In 47 of the tests, the heart stopped for an average time of 7 sec.



Change in frog heart rate on stimulating thigh muscles. a) Twice-distilled water applied to the muscles; b) muscles irrigated with twice-distilled water. Arrows indicate onset (↑) and end (↓) of stimulation.

Proprioceptor stimulation with hyper- and hypotonic solutions, therefore, usually causes marked changes in the amplitude of the cardiac contraction or in the heart rate. Similar results [2] were obtained by stretching the muscles.

It can be seen, therefore, that muscular stimulation by either hyper- or hypotonic solutions will cause different degrees of irradiation according to the strength.

The heart rate is affected only when proprioceptor stimulation is intense. The threshold was higher for this response than for the alteration in the amplitude of the contractions.

The threshold for the motor response was higher still. In our experiments, the response took different forms: there might be a fibrillation of the muscle at the point to which the stimulus was applied, or a movement of the digits or of the whole limb, or possibly a generalized motor response. The latent period for this reaction was as a rule longer than that for the cardiac response.

The characteristic stepwise relationship between the cardiac and skeletal-muscle responses to proprioceptor stimulation should be noted. A. A. Ukhtomskii's principle of stepped theresholds applies to interoceptors as well as proprioceptors [8].

The principle of the effect of hypertonic sodium chloride solutions and of distilled water on propioceptors is as follows.

Disturbance to the vital function of an organ or tissue may be brought about either by a change of salt content or of the osmotic pressure of the solution. However, it has been shown [12], that the osmotic factor acts first. Only after a longer time interval is the effect due

to diffusion of salts reduced [10]. In our experiments the time of action of the solutions was limited to 1 ½-3 min, and it is probable, therefore, that proprioceptor impulses were induced by increase or decrease in osmotic pressure.

When the muscles were irrigated with twice-distilled water for periods up to 3 min, the initial cardiac change was not maintained for the whole period. Evidently, the considerable reduction in osmotic pressure first stimulates and then depresses the proprioceptors. It is known [4] that distilled water causes some loss of both motor and receptor muscle-fiber function.

In our method, repetition of the irrigation with distilled water after an interval of 5-10 min again caused an irradiation of the response affecting heart and skeletal muscle. Evidently, the suppressor effect of osmotic pressure changes on muscle receptors is rapidly removed by the mechanism for regulating the osmotic pressure.

The cardiac and skeletal muscle changes induced by the action of hyper- and hypotonic solutions on muscles takes the form of a motor visceral reflex. The latent period has an average value of 12-16 sec. The latent period depends on the rate of diffusion of the water. The reflex nature of the changes is shown by the fact that in 50 tests, the reaction occurred 2-7 sec after applying the sodium chloride solution or water to the muscle.

Proprioceptors are therefore sensitive to osmotic pressure changes so that reflexes involving responses of viscera or skeletal muscles may be produced not only mechanically but also by physicochemical stimuli.

SUMMARY

Experiments were performed on bulbar frogs. Ringer's solution, 0.65 % physiological saline, 1.3 and 1.95 % sodium chloride solutions, and distilled water were applied to the femoral muscles for periods ranging from several seconds to three minutes. The solutions were applied on filter paper, or else were used to irrigate the muscles. It appeared that the induced change of proprioceptor osmotic pressure constituted a physicochemical stimulus which provoked a reflex cardiac change. In most cases the heart beat slowed or stopped, and the amplitude of contractions diminished.

LITERATURE CITED

1. R. A. Arutyunyan and R. A. Durinyan, Collection of Students' Work at the Eleventh All-Union Congress of Students, Scientific Societies, and Medical Correspondence Institutes [in Russian] (Moscow, 1954) pp. 14-17.
2. V. I. Bel'tyukov, Byull. Éksp. Biol. i Med. No. 7, 47 (1947).
3. I. D. Boenko, Collection of Scientific Works of the Chitinsk Medical Institute [in Russian] (Chita, 1957) pp. 33-42.
4. G. V. Gershuni and S. P. Narikashvili, Doklady Akad. Nauk SSSR, 36, 123-128 (1942).
5. T. P. Dmitrieva and M. R. Mogendovich, Doklady Akad. Nauk SSSR, 111, 914-916 (1956).
6. V. P. Kolychev, The Mechanisms and Path or Propioceptor Regulation of Certain Somatic and Autonomic Functions Candidate's dissertation) [in Russian] (Perm', 1959).
7. V. R. Maisaya, Byull. Éksp. Biol. i Med. No. 10, 48-52 (1955).
8. M. R. Mogendovich, Reflex Interaction of the Locomotor and Visceral Systems [in Russian] (Leningrad, 1957).
9. V. I. Nikoliev, Reports and Abstracts of the Second Ural Conference of Physiologists, Biochemists, and Pharmacologists [in Russian] (Perm', 1957) pp. 51-53.
10. D. L. Rubinstein, General Physiology [in Russian] (Moscow, 1947).
11. G. E. Skachedub, Contributions to the Physiology of the Internal Analyzors (Candidates dissertation) [in Russian] (Perm', 1953).
12. B. N. Tarusov, Zhur. Éksp. Biol. i Med. No. 6, 144-154 (1926).
13. V. A. Fedorova, quoted by M. R. Mogendovich.
14. Frey, quoted by M. R. Mogendovich.
15. Loeb, quoted by D. L. Rubinstein.
16. B. H. C. Matthews, J. Physiol. 71, 64 (1931).
17. E. Overton, Arch. ges. Physiol. 92, 346 (1902).

THE ACTION OF ADRENALIN ON THE INTERNAL CAROTID AND VERTEBRAL ARTERIES

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By studying several indices of cerebral circulation we have been able to show [3] that the internal carotid and vertebral arteries play an important part in compensating for disturbances of the cerebral circulation resulting from interference with the outflow of blood from the brain. By fixing the arteries in vivo we were also able to reveal in which parts the "closure mechanisms" which control the flow of blood to the brain were localized. After further work we concluded that the simplest and most convenient way of determining the condition of these arteries was to determine directly the pressure drop (resistance) in them, because it is known that this quantity depends chiefly upon the size of the lumen; when the vessel is contricted the resistance increases, and vice versa. By measuring the pressure at both ends of the arteries, the aperture of the lumen may be determined.

In the present work we have studied the effect of adrenalin on the internal carotid and vertebral arteries. This action is important because adrenalin is one of the most widespread physiologically active substances and is concerned in the regulation of many functions including blood circulation. In addition, adrenalin or compounds closely related to it mediate the transmission of sympathetic nervous impulses, and our study may help to determine the effect of the sympathetic nervous system on the arteries supplying the brain.

METHOD

The experiments were performed at operation upon 13 adult rabbits. In some experiments the anesthetic used was chloral hydrate (0.4 g/kg), and in others urethane (1 g/kg). After operation, 0.3-0.4 ml/kg heparin was injected.

To determine changes in the size of the lumen, simultaneous recordings were made of the blood pressure in the aorta and in its major branches from which the vertebral and internal carotid arteries originate, and also in the circle of Willis, where they end. For this purpose it was necessary to ligature one of the carotid arteries, a procedure which does not affect the cerebral circulation [5]. A cannula was inserted in the direction of the thorax into the common carotid artery, and the pressure in the aorta and its major branches was recorded on a mercury manometer. A second cannula inserted into the same carotid artery in a cranial direction enabled the pressure in the circle of Willis to be recorded by the method previously described by Hürthle [6] and Opdyke [7] and Avrorov [1]. To increase the accuracy, we tied off all the branches of the artery being investigated except for the internal carotid, which was dissected out up to the point at which it enters the skull, and this artery was then effectively a continuation of the cannula and connected it directly with the circle of Willis. Before the experiment, both manometers were arranged so that their zero points were at the same level.

In some experiments, a compensator was used which enabled the aortic pressure to be maintained at a constant level. It consisted (Fig. 1) of a manometer, an air reservoir, and a vessel containing a blood substitute * which was connected by a wide cannula to the abdominal aorta below the renal arteries. In the compensator, the pressure was maintained at the same level as in the aorta. The compensator automatically corrected for any increase or decrease in arterial pressure.

RESULTS

It is known that even quite small doses of adrenalin cause an increase in arterial pressure. It is difficult to determine what effect it has on the circulation of the brain as a whole and on the flow through the carotid and vertebral arteries in particular, because any increase in arterial pressure will have a marked influence on the arteries of the brain. It is therefore not possible to determine whether changes in the cerebral circulation are caused by the direct action of adrenalin on the arteries or whether they are the result of increased arterial pressure. On this account we maintained the arterial pressure at a constant level by means of the compensator, as has been described by Carlyle and Grayson [5], and by Thuranszky [8].

* The most suitable fluid was polyglucin, which has no antigenic properties.

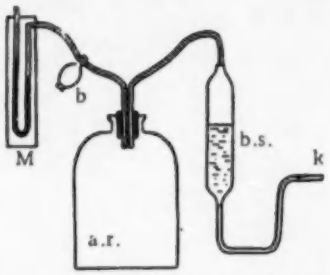

Fig. 1. Diagram of the compensator used to maintain arterial pressure. k) Cannula inserted into large artery and directed towards the heart; b.s.) blood substitute; a.r.) air reservoir; b) bulb with valve; M) manometer for measuring the pressure in the whole system.

When adrenalin is injected subcutaneously, it is absorbed slowly and at a rate which will depend upon the conditions. However, an intravenous injection will cause the pressure to rise so rapidly that it cannot be annulled by the compensator. We therefore injected $1 \cdot 10^{-4}$ adrenalin slowly into the peritoneal cavity. This enabled a comparatively slow increase of adrenalin in the blood to occur, and at the same time the compensator was effective in maintaining the arterial pressure constant.

The results of one experiment are shown in Fig. 2. In spite of the fact that there was no change in aortic pressure during the injection (upper curve), the pressure in the circle of Willis fell considerably (lower curve). This constitutes a direct demonstration of the increase in resistance offered by the internal carotid and vertebral arteries. Because there is no reason to expect any changes in blood viscosity, the effect observed can result only from the constriction of these arteries under the influence of adrenaline. The pressure between the two ends of these vessels, which indicates the increased resistance, was approximately doubled (Fig. 2, shaded area). The constrictor effect of adrenalin on these arteries is quite specific, because in our experiments it was eliminated by the adrenolytic substance dibenamine (TS-224).

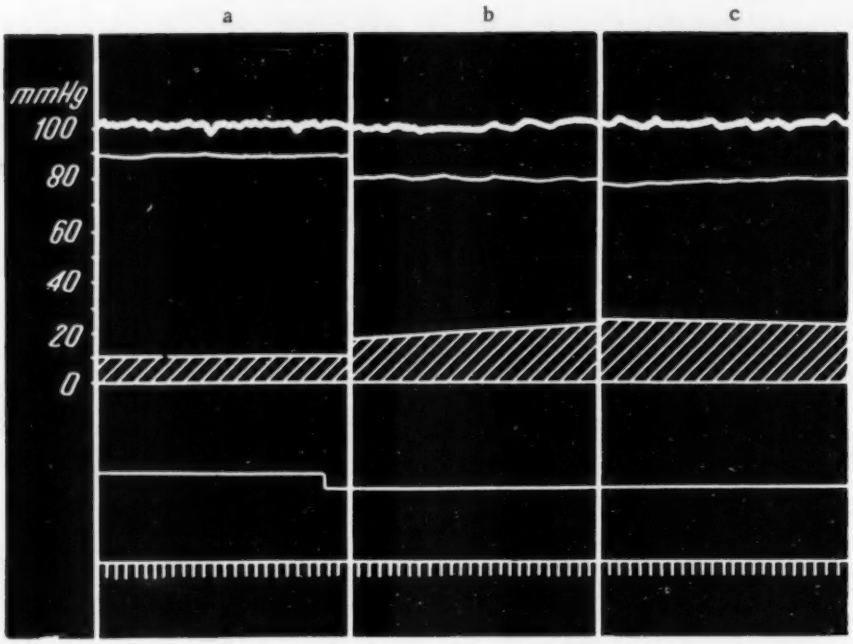

Fig. 2. Increase in the resistance of the internal carotid and vertebral arteries following slow intraperitoneal injection of $1:10^{-4}$ adrenalin. Compensator used for arterial pressure. Upper curve –pressure in aorta; lower curve– pressure in circle of Willis; shaded area over the base line represents the difference of the two pressures and represents the resistance in the internal carotid and vertebral arteries; time marker–5 sec; a) Before the injection; b) ten minutes after the beginning of the injection. 18 min after beginning the adrenalin injection.

The action of adrenalin on the internal carotid and vertebral arteries was also observed after giving small repeated intravenous injections. The results of one such experiment are shown in Fig. 3. The elevation of arterial pressure (upper curve) was approximately the same for each adrenalin injection. However, if at the first injection the pressure in the circle of Willis (lower curve) increased beyond a certain level, then the second elevation was less well shown, and subsequently scarcely occurred at all. Such an increase in the difference between the pressures at the beginning and end of the arteries indicates their constriction. Therefore, to demonstrate the action of adrenalin on the internal carotid and vertebral arteries, either a larger dose or a longer time of action of adrenalin, is required. At the end of the experiment, as a control, a rather greater amount of adrenaline was injected, and this caused a greater increase in general arterial pressure and in the circle of Willis than previously.

Hürthle [6] was the first to make simultaneous measurements of the pressure in the aorta (m_1) and in the circle of Willis (m_2) and he thought that from the ratio $m_2/m_1 = \omega$ it would be possible to determine the condition of the arteries supplying the brain. His method is based on a model consisting of a right-angled triangle where one of the sides enclosing the right angle represents the vertical manometer tubes, and the hypotenuse represents the pressure gradient in a horizontal tube which forms the other side enclosing the right angle. The ratio m_2/m_1 then represents the resistance in the horizontal tube only in the case when there is a linear pressure gradient, which however is not the case in a vascular system. Hürthle himself [6] admitted that the method could be applied to the blood system of the brain only under the conditions that the resistance in the blood vessel connecting the manometers (i.e., in the arteries supplying the brain) remained constant; actually, however, the lumen of these vessels and of all their branches may undergo considerable changes.†

It is quite evident that the readings of two manometers placed at different points of the vessels can indicate only the resistance between them, so that the resistance would be represented by the difference in

† Hürthle's method has not been in common use for 70 years, and we would not have discussed it had not V. P. Avrorov [2] used it to explain his experimental results.

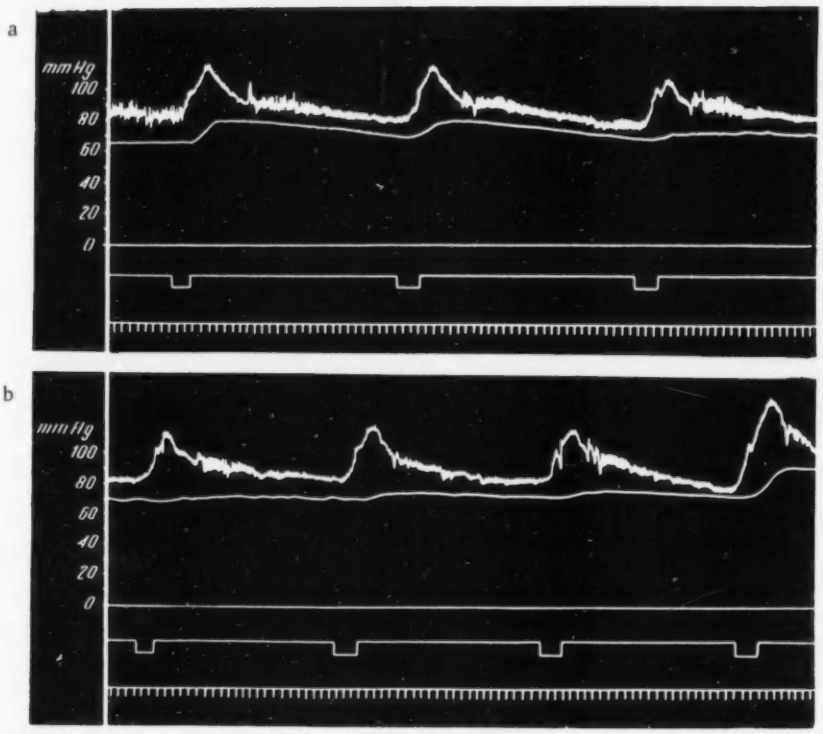

Fig. 3. Increase in the resistance of the internal carotid and vertebral arteries following repeated intravenous injections of 0.1 ml 1 : 10^{-4} adrenalin; the seventh injection is of 0.2 ml. Upper curve) pressure in aorta; next curve) pressure in the circle of Willis, then follow : zero line, marker indicating adrenalin injection, 5 sec time marker; b represents a direct continuation of trace a. Explanation in text.

pressure and not, as proposed by Hürthle, by the ratio m_2/m_1. If deductions are to be made from this ratio, then the results shown in Fig. 2 would have to be interpreted as indicating an expansion of the blood vessels. However, our direct measurements showed this conclusion to be erroneous, because micrographs of the pial vessels and measurement of their diameter on the film made with a micrometer microscope eyepiece showed that not only did the superficial arteries not expand, but that they were actually constricted by 4-5 %. At the same time the pressure in the venous sinuses of the brain fell somewhat.

The results of the present experiments allow the value of the resistance in the internal carotid and vertebral arteries to be determined. Under normal conditions (without adrenalin) the pressure drop represents approximately 12 % of the aortic pressure, and when adrenalin is injected the figure is 26 %, i.e., more than one quarter of the aortic pressure. These arteries may therefore represent a considerable resistance to cerebral blood flow, and this resistance is increased by adrenalin. The effect of adrenalin on the internal carotid and vertebral arteries, which we have shown to affect the blood flow to the circle of Willis, explains on the one hand certain aspects of the action of this physiologically active substance on the cerebral circulation, and on the other hand affords (admittedly indirect) evidence that the lumen of these arteries is under sympathetic control. The second point awaits direct experimental confirmation.

SUMMARY

Experiments were performed on rabbits. Measurements were made of the difference in pressure between the aorta and the circle of Willis, and the resistance of the internal carotid and vertebral arteries deduced. When the arterial blood pressure was stabilized by means of a compensator, it was shown that slow intraperitoneal injection of adrenalin caused a considerable constriction of these arteries. A similar reaction occurred following multiple intravenous adrenalin injections, and the pressure in the circle of Willis failed to follow the rise in aortic pressure.

It was shown that adrenalin injection caused a significant increase in the resistance of the internal carotid and vertebral arteries.

LITERATURE CITED

1. V. P. Avrorov, Byull. Éksp. . Biol. i Med. 44, 11 (1957).‡
2. V. P. Avrorov, Fiziol. Zhur. SSSR 44, 404 (1958).
3. G. I. Mchedlishvili, Fiziol. Zhur. SSSR 45, 1221 (1959).
4. G. I. Mchedlishvili, Doklady Akad. Nauk SSSR 124, 1371 (1959).‡
5. A. Carlyle and J. Grayson, J. Physiol. 133, 10 (1956).
6. K. Hürthle, Arch. ges. Physiol. 44, 561 (1889).
7. D. F. Opdyke, Am. J. Physiol. 146, 467 (1946).
8. K. Thuranszky, Der Blutkreislauf der Netzhaut (Budapest, 1957).

‡ Original Russian pagination See C.B. Translation.

CHANGED BLOOD SUPPLY IN THE FINGERS OF AN INACTIVE HAND FOLLOWING RAISING OF A LOAD WITH THE OTHER ONE

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Many authors [1, 9, 10, 15] have shown that the blood supply to both arm and leg in man increases immediately at the end of physical exercise.

Weber [12-14] examined several thousand healthy human subjects and found an increased blood supply in an idle limb while work was being performed by the other. It was shown that movements of the foot in an unfatigued resting individual cause an increased blood flow to the hand, which falls away rapidly when the exercise is stopped.

Plethysmography of the limbs during physical work indicates the state of the blood supply to the skin and muscle, but chiefly to the muscle. Until now, insufficient information concerning the blood supply to the skin has been obtained.

In studies on 3 healthy men [11] it was shown that work performed by the legs on a bicycle-ergometer causes an increased blood supply both to the muscles of the forearm and to the subcutaneous tissue of the hand, the latter increase being the greater.

On the other hand, it has been shown [3] that when one arm is performing work there is a reduction in the number of functional capillaries of the idle arm. It has also been observed [2] that after fatigue has been induced in the hand, a spasm of the cutaneous capillaries may occur.

The present investigation was undertaken in order to explain certain changes in the digital circulation of one hand following cessation of work with the other.

METHOD

Plethysmographic recordings were made from the terminal phalanx of a finger of the left hand before and after physical work.

To determine the nature and extent of the peripheral circulation we employed the method proposed in 1907-1909 by M. V. Yanovskii and A. I. Ignatovskii [4], and also by Hewlett and Van Zwaluwenburg [7]; this method consists in blocking the venous return from the hand while maintaining the arterial supply (the method of venous occlusion plethysmography).

The main advantage of this method is that it allows both the initial flow rate and its change in response to any stimulus to be determined.

It has been shown in experiments on dogs [8] that the values obtained by plethysmography with arrest of venous outflow are the same as those obtained when an electromagnetic flowmeter is used. Modern recording techniques and the method of evaluating the curves to a large extent make good the defects indicated by V. A. Val'dman and D. M. Abdulaev [1] and by other investigators.

In each subject the blood pressure was obtained while he was in the sitting position, by means of a mercury sphygmomanometer, and the plethysmograph was attached to the terminal phalanx of the finger and smeared with vacuum grease at the junction (Fig. 1A). Every minute the plethysmogram was recorded on film during a 20-second compression of the veins of the upper arm which was effected by rapidly compressing the air in the cuff to the diastolic pressure.

After 3-4 such measurements had been made, recording was discontinued and the subject was told to raise a weight of 5 or 8.5 kg with this right hand to the level of the axilla. The work was continued until the subject failed from fatigue. Thirty seconds after the end of the working period, veneous outflow was again blocked and the plethysmogram recorded, after which 2 or 3 recordings were made at one minute intervals. By this time, in most cases, the blood flow to the finger had returned to the initial rate.

To calibrate finger volume changes, in 11 human subjects, after the plethysmogram had been recorded, 0.02, 0.04, 0.06, 0.08, and 0.1 cm^3 of air were injected from a tuberculin syringe into the closed system. The volume of the phalanx of the finger from which the plethysmogram was obtained was determined by means of a measuring cylinder.

A diagram illustrating a plethysmograph curve is shown in Fig. 1b.

From the height h_1, the rate of blood flow to the finger phalanx was calculated, and from h_2 the average rate could be obtained. The increase in the initial rate after physical work is indicated by a plus sign (positive vascular response) and a reduction is shown by a minus sign (negative response); a zero indicates no response. The same indications were used to convey changes in the average rate of blood flow.

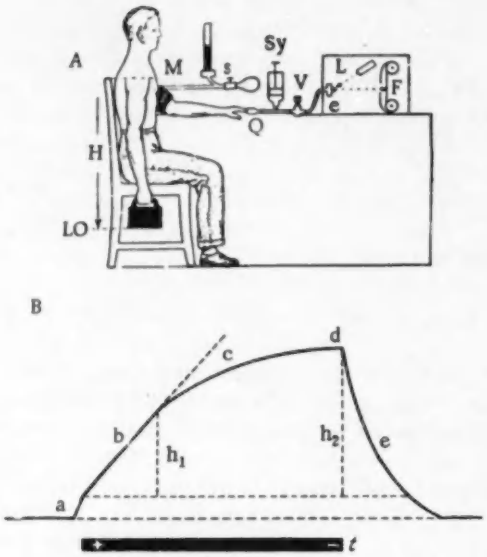

Fig. 1. Diagram of the apparatus (A) and the type of curve obtained for the blood flow to a finger phalanx (B), obtained by compressing the veins of the upper arm for a short time. Diagram A : S) Sphygmomanometer; M) cuff of sphygmomanometer; P) plethysmograph; V) valve for opening and hermetically sealing the transmission system; C) capsule of plethysmograph; Sy) 20 ml syringe for changing the volume (sensitivity) of the transmission system; L) lamp; F) film; H) height of 60 cm to which load (Lo) is raised. Diagram B: a) Artifact caused by inflation of cuff; b) initial change in volume of phalanx; c) subsequent increase in blood filling; d) no further change in volume of phalanx occurs; e) fall in curve following release of air from cuff; t) duration of occlusion of veins of upper arm (20 sec).

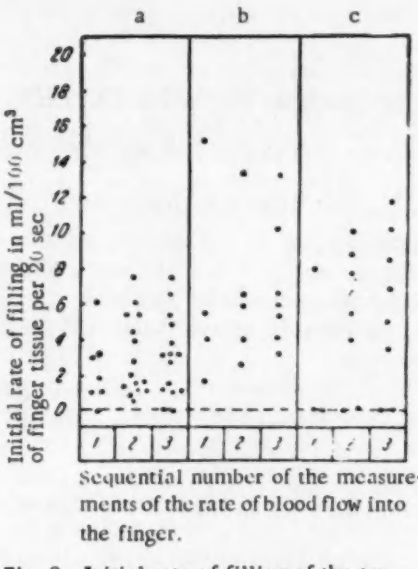

Fig. 2. Initial rate of filling of the terminal finger phalanx in subjects tested at rest after temporarily blocking the venous outflow. a) When a positive response to physical work was obtained; b) when a negative response occurred; c) response zero. Each point on the graph corresponds to the blood flow rate as determined in one measurement on one subject before beginning the exercise. A) Initial rate of filling in ml / 100 cm^3 of finger tissue per 20 sec; B) sequential number of the measurements of the rate of blood flow into the finger.

Nineteen healthy people comprising 14 women and 5 men aged from 18 to 49 years were investigated; none had a history of any cardiovascular or other disorder. Most were laboratory assistants, nurses, or technicians.

RESULTS

After spending 10 minutes in becoming accustomed to the surroundings, the baseline plethysmogram at rest was "zero" or else showed some slight variation. The last 2 or 3 recordings before the beginning of physical exercise were quite consistent.

Results on the initial rate of filling of the terminal finger phalanx are shown in Fig. 2, where it can be seen that the initial rate at rest as a rule varied between 1 and 8 ml of blood per 20 seconds; the volume of the tissue of the finger was 100 cm^3. This flow rate represents 3-24 ml of blood per minute. These values lie within the published figures for the blood flow rate to the skin and underlying tissue [5, 6].

Only in 4 subjects at rest was there a transitory (Kon-a, Sam-n) or maintained (G-a) spasm of the skin vessels; occluding the venous outflow caused no change in the rate of filling of the terminal phalanx. In addition, a forced voluntary inhalation caused either a very small or no vascular reaction. Of the 4 subjects tested, an initial filling rate between 24 and 60 ml of blood per min per 100 cm^3 of finger tissue was found in 3 only (Sm-a, A-b, Art-a).

In most cases, after physical work the plethysmogram showed changes in the blood supply to the fingers of the opposite hand (see Table).

In 12 subjects, the physical exercises caused mainly an increase in the initial rate of blood filling; in seven the flow was either reduced or unchanged.

Of the 54 tests, there was an increased blood inflow after exercise in 34, and in four of these there was a first a transitory reduction. A reduced flow was observed in 11 cases, and in nine there was no change.

Therefore the characteristic response of the finger vessels of one hand when a load is lifted by the other, is that the blood inflow increases (Fig. 3). Under certain

Changes in Rate of Blood Supply to the Fingers After Physical Work

No. in sequence	Surname of subject	Sex	Age (in years)	Air temp	Number of tests	Physical exercise			Pulse rate in beats per min		Principal change in the rate of blood inflow		Remarks
						weight of load (in kg)	number of times load was raised	duration of exercise (in seconds)	before exercise	after exercise	initial rate	mean rate	
1	D-a	F	24	18—22°	5	8,5	15—30	Not measured	72—88	88—100	+	+	
2	T-a	F	49	24°	1	8,5	20	» »	48	60	+	⊖	
3	B-v	M	25	20—22°	3	8,5	40—45	» »	64—68	76—80	+	+	Variable baseline
4	St-a	F	21	20°	2	8,5	10—20	» »	80—96	96	+	+	
5	R-a	F	21	19—22°	4	8,5	7—15	» »	68—76	68—76	+	Not measured	Variable baseline
6	Sp-n	M	26	18—22°	3	8,5	40—45	45—60	40—52	44—56	⊖*	⊖	
7	Bu-a	F	25	18—19°	3	8,5	19	25—30	66—76	80—88	+	+	
8	Ch-o	F	29	18—19°	3	8,5	20—25	55—60	64—68	68—70	—	—	Subject embarrased and blushes
9	Aru-a	F	24	22—23°	3	5	24—30	30—50	76—80	76—92	+	+	
10	Ku-a	F	39	23°	1	5	17	30	92	88	—	—	Refused further tests
11	Sm-a	F	20	22,5—23°	3	5	9—14	20—35	72—80	72—92	⊖	+	
12	A-v	M	25	18—21°	4	8,5	27—35	85—110	76—96	104—112	⊖	+	
13	Art-a	F	38	21,5—23°	3	5	11—20	13—25	68—88	72—80	⊖	⊖	
14	G-a	F	20	19—23°	3	5	9—16	15—30	72	72	⊖	⊖	Maintained spasm of finger vessels
15	Kis-a	F	18	18—19,5°	3	5	18—21	35—45	72	72—76	+	⊖	
16	K-v	M	29	19—20,5°	3	8,5	25	50—65	72	72	+	+	
17	Kon-a	F	28	18,5°	1	5	15	35	Not measured	80	+	+	
18	Sam-n	M	21	19—20°	3	8,5	20	45—50	76—80	76—84	+	+	
19	Bar-a	F	19	21—22°	3	5	10—18	30—45	80—92	88—96	+	⊖	

* The sign ⊖ indicates that mainly negative or zero responses were obtained .

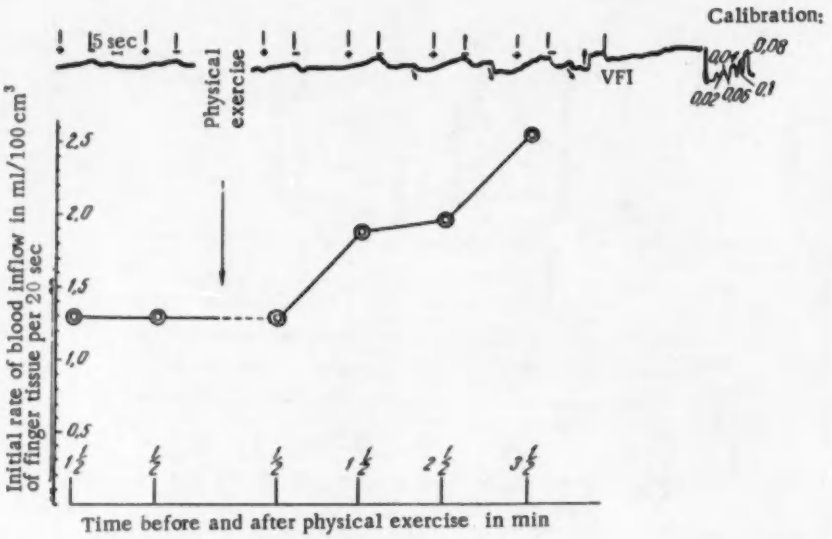

Fig. 3. Change in the initial rate of blood inflow into the terminal phalanx of one finger after raising a load with the other hand. Subject K-v. Experiment October 23,1956 at 10.56 a.m. Air temperature 19°. Load of 8.5 kg raised to height of 60 cm by the right hand 25 times in 65 min. ↕) Indicates raising the pressure in the cuff to 75 mm Hg; ⊥) moment when pressure is reduced to zero; ↑ ↓) displacement of recording; VFI) voluntary forced inspiration. (1) Initial rate of blood inflow in ml / 100 cm³ of finger tissue per 20 sec; (2) time before and after physical exercise, in min; (3) calibration : 0.04 ml; (4) physical exercise.

conditions which were not easy to determine it might happen that the response was abnormal or inhibited. Some of these cases may have been due to a high initial finger-flow rate (Fig. 2 b and c). The emotional condition of the subjects must also be taken into account (see Table , Ch-o, Ku-a).

No relationship between the vascular response and the change in pulse rate was observed. Positive, zero,or negative vascular reactions occurred together with a slowing or with a considerable increase in pulse rate .

As a rule, a high initial flow was associated with a greater average flow rate. After physical exercise, the change in average rate did not always correspond to the change in the initial rate of filling, and the reason was probably that there was some variation in the volume of the venous bloodstream in the finger.

Thus, when raising a load with one hand, in the other, besides an increase in the blood supply to the muscle, there is also an increased flow to the skin. According to published results [3], when this occurs the capillary flow is reduced. It must therefore be supposed that when work is performed with one hand the skin flow to the other is increased chiefly by an increased passage of blood through the shunting arteriovenous anastomoses.

SUMMARY

Venous occlusion plethysmography was used to study changes occurring in the finger circulation after physical exercise.

In healthy individuals, after raising a load with one hand there was usually an increased blood supply to the skin and subcutaneous connective tissue in the fingers of the other.

LITERATURE CITED

1. V. A. Val'dman and D. M. Abdulaev, Ter. arkh. 6 , 237 (1928).
2. E. O. Teplitskaya, Problems of Physiology [in Russian] (Kiev, 1953), No. 3, p. 135.
3. S. I. Fudel'-Osipova, Fiziol. Zhurn. SSSR 30 , 574 (1941).
4. M. V. Yanovskii and A. I. Ignatovskii, Izv. Imper. Voen.-med. Akad. 14 , 287 (1907).
5. A. C. Burton, Am. J. Physiol. 127, 437 (1939).
6. K. E. Cooper, O. Edholm, and R. F. Mottram, J. Physiol. London 128 , 258 (1955).
7. A. W. Hewlett and J. G. Van Zwaluwenburg, quoted by A. C. Burton.
8. C. J. Imig, D. C. Sutfin, and H. M. Hines, Am. J. Phys. Med. 34 , 355 (1955).

9. P. I. Korner, Austral. J. Exper. Biol. and Med. 30, 375 (1952).
10. A. H. G. Love, J. Physiol. London 127, 13P (1955).
11. R. Ruosteenoja, Acta Physiol. Scand. 31, 248 (1954).
12. E. Weber, Arch. Anat. Physiol. p. 290 (1914).
13. E. Weber, Dtsch. med. Wschr. 48, 894 (1922).
14. E. Weber, Spezielle Pathologie und Therapie innerer Krankheiten. (Berlin, 1925), p. 334.
15. L. H. Wisham, R. S. Yalow, and A. J. Freund, Am. Heart. J. 41, 810 (1951).

THE RELATION OF HUMAN VASCULAR REFLEXES TO THE TYPE OF HIGHER NERVOUS ACTIVITY

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The particular type of higher nervous activity characteristic of an individual is of considerable importance in the practice of medicine, teaching, psychology, and other sciences. Long ago S. P. Botkin [1] observed a relationship between the course of an illness and the condition of the central nervous system. In many Russian clinics (M. V. Chernorutskii [9], K. G. Nikulin [2], I. I. Speranskii [5], and others), various means are being used to determine the type of higher nervous activity of the patient in order to enable more effective therapeutic methods to be applied.

In the present work we have attempted to apply the method of vascular reflexes to determine objectively some typological features of human higher nervous activity.

It is not by chance that the vascular reaction has been chosen as an index of the cerebral nervous processes. K. M. Bykov and A. A. Rogov [4], and A. T. Pshonik [3] have shown that there is a very close relationship between the two phenomena. Several authors [3, 4, 6, 7] who have used the plethysmographic method have demonstrated the influence of cortical processes on the curve obtained.

METHOD

Our observations were made on 70 healthy human subjects who were maintained under almost uniform living conditions, nutrition, and work. A study was made of their personal characteristics and family background, and observations were made on their behavior. The personal details were drawn up according to the scheme developed by Professor F. P. Maiorov. The vascular reaction was recorded plethysmographically. The correlation of the objective vascular measurement with the personal data represented a qualitative study of human higher nervous activity.

Measurements were made of the vascular reflexes as affected by orienting reactions and by conditioned and unconditioned stimuli.

The unconditioned stimuli used were cold or heat applied to the forearm of the limb on which the measurements were made. In addition, a powerful buzzer and a weak bell were used as remote stimuli. The unconditioned stimuli were applied for 30 sec at intervals of 5-6 min.

The conditioned reflex was developed by A. T. Pshonik's method. The essential feature of this procedure is that by using the second signalling system which is characteristic of man, the experimenter informs the subject about the relationship between the conditioned and unconditioned stimuli, and thereby makes it unnecessary to present the two stimuli together a large number of times. As a result, the conditioned reflex in a healthy subject is formed at the first combined presentation.

The principal vascular conditioned reflexes were those obtained in response to "buzzer + cold" and "bell + heat". The combined stimuli were applied at intervals of 5-6 min, and established the first set of conditioned reflexes. The second pair of stimuli represented a reversal of the first, so that instead of "buzzer + cold", "buzzer + heat" was presented, and likewise "bell + cold" was substituted for "bell + heat". The unconditioned stimulus was presented 30 sec after the onset of the conditioned signal, and the two were continued together for 30 sec.

RESULTS

The plethysmographic curves obtained gave some indication of the typological features of human higher nervous activity.

A physiological analysis of the orienting reflex is very important in connection with higher activity types. Until now, insufficient attention has been paid to this reaction, but nevertheless the orienting reflexes represent the most numerous and biologically important class.

From the effect of orienting reactions on the plethysmographic curves and from observations on the pulse rate we were able to distinguish individuals having different types of higher nervous activity. Thus, for instance, for subjects with a strong and balanced type of nervous system, the plethysmogram curve is undulating, and rapidly passes over to the "zero" type. This is shown

in the curves of the subject K-a, aged 24, which are shown in Fig. 1, a, b. It can be seen that third order waves are present, and these give the plethysmogram its undulating appearance. When further traces are recorded, the third order waves gradually disappear. By the end of the experiment, the plethysmogram had changed into the "zero" type. This result indicates the rapid adaptation of the individual to the laboratory conditions.

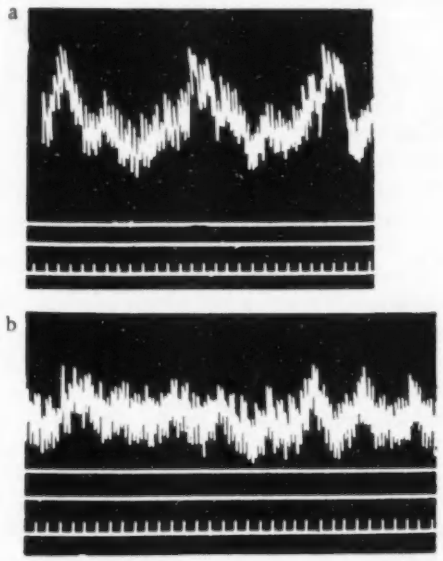

Fig. 1. Human plethysmograms. a) Undulating type; b) "zero" type. Curves from above, downwards: vascular response; conditioned stimulus; unconditioned stimulus; time marker.

In subjects having a strong unbalanced type of nervous system, the undulating type of curve was most common, and as a rule these did not pass rapidly into the "zero" type. A "zero" plethysmogram might be obtained sometimes, but it would then change back to the undulating type at the next test; the curves show a rhythmical pulse wave.

In subjects with a weak type of nervous system, the vascular responses are mostly arrhythmical; the variations occur on both sides of the baseline. A typical curve is that of B-i, aged 22 (Fig. 2). In this curve the undulations and irregular rhythm can be clearly seen, and there are sudden drops and more sudden elevations of the curve, which resemble a motor response. These typical irregularities were not observed in any members of the strong type of nervous activity.

Because of the multiplicity of vascular responses in human subjects having different types of higher nervous activity, it is not sufficient to determine the type to which the individual belongs in terms of the orienting response. For a complete assessment, account

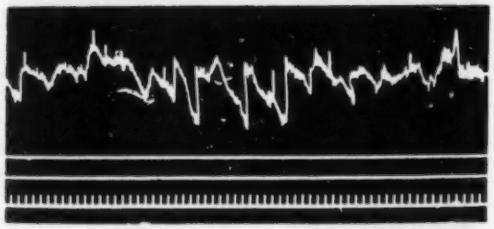

Fig. 2. Arrhythmical undulating plethysmogram. Curves from above, downwards: vascular reaction; conditioned stimulus; unconditioned stimulus; time marker.

must be taken of vascular reactions obtained in response to both conditioned and unconditioned stimuli .

A. A. Rogov [4], A. T. Pshonik [3], and others have shown that at the first stage of an investigation the vascular response to any unconditioned stimulus consists of a constriction of the blood vessels, so that the plethysmogram curve falls. Differentiation of the vascular reaction to correspond with the stimulus occurs much later. This result was completely confirmed in our tests. It is true that the vasoconstrictor response to the unconditioned stimulus was not the same in all subjects: in some it was better shown than in others, while in certain cases it was masked. We have found that these differences in the reactions depend on the different types of higher nervous activity.

In subjects of the strong and balanced type, the vascular response to unconditioned stimuli is well shown, and in the first stage, the vasoconstrictor response predominates. In the strong unbalanced type, the vascular response to the unconditioned stimulus is masked by the undulations in the curve. The preponderance of the inhibitory processes has the effect that there is no reaction to the unconditioned stimulus. In subjects having the weak type of nervous system, as a rule, the responses are irregular and complicated by motor reactions.

I. P. Pavlov's work on types of nervous systems was based on the study of conditioned reflexes. His principal task was to study higher nervous activity in man. The first human conditioned vascular reflex and its differentiation was effected by I. S. Tsitovich [8] in 1918.

In subjects with the strong and balanced type of nervous system, as a rule, the vascular responses were well developed. Figure 3 shows the plethysmogram of R-v, aged 24, who was of this type. There was a well developed vascular reaction in response to the combination "buzzer + cold"; the vessels contracted sharply and almost immediately in response to the conditioned stimulus, and there was a further contraction to the unconditioned stimulus. Here, the vascular response was superimposed on the original "zero" plethysmogram; there was considerable variation in pulse amplitude. The curve was obtained on the first day, in response to the second application of the signals as described above.

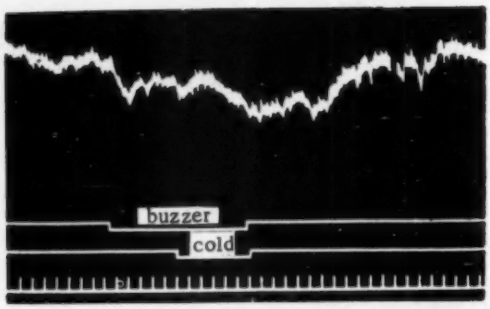

Fig. 3. Vascular conditioned reflex in response to "buzzer + cold". Curves from above, downwards: vascular response; conditioned stimulus; unconditioned stimulus; time marker(5 sec)

This marked vascular constriction developed immediately after verbal instruction concerning the combination of stimuli to be presented.

When the inhibitory process preponderates, conditioned vascular reflexes are inhibited.

In subjects of the strong and unbalanced type, conditioned reflex responses are often undulating. In subjects with a weak type of nervous system, they quite frequently fail to develop, or else develop paradoxically, with irregular pulse variations and with superimposed motor responses. Verbal instruction may elicit a vascular response, but sometimes there is none. Consider the plethysmogram of K-v, aged 29, who had a weak type

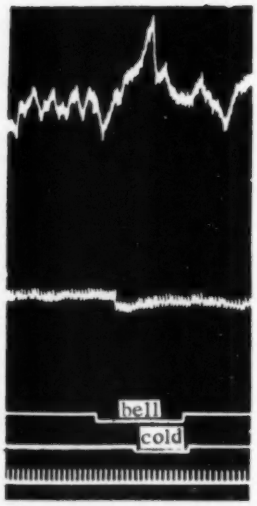

Fig. 4. Paradoxical conditioned vascular reaction to the combination "bell+ cold". Curves from above, downwards: vascular reaction; pneumogram; conditioned stimulus; unconditioned stimulus; time marker (3 seconds).

of nervous system (Fig. 4). It can be seen that there is a paradoxical conditioned vascular reaction to the combination "bell + cold". The plethysmogram shows irregular pulse fluctuations with some small superimposed motor effects.

A careful analysis of the vascular reactions occurring in the experiments just described showed that in each case the functional condition of the cerebral cortex could be determined. When a number of recordings of the vascular responses of a subject at rest are made, if the cortical processes are equilibrated, the undulations in the curve usually cease. The degree of equilibration will be indicated by the rate at which the undulations die away. When they persist, unbalanced cortical processes are indicated. In subjects with the weak type of nervous system, the vascular responses are usually irregular, and the deviations occur on both sides of the baseline.

We took as normal the vascular response of a subject having equilibrated cortical processes.

When the vascular response to unconditioned stimuli is better developed than normally, and when it is maintained for a considerable time without returning to the initial level, then in the individual the excitatory process preponderates over the inhibitory. If in response to the unconditioned stimulus, the vascular change is indefinite or absent, then the inhibitory process preponderates over the excitatory. Usually, in subjects of the strong and balanced type, unconditioned vascular responses are well shown and consist in a constriction of the blood vessels. An unconditioned vascular reflex in subjects of the strong unbalanced type of nervous system produces a plethysmogram in which the undulations are maintained up to the time of the application of the unconditioned stimulus, and they continue after it has been withdrawn. Irregular pulse beat and motor responses are characteristic for the unconditioned vascular reflexes in subjects having the weak type of nervous system. In people of this type, there is not always a definite constriction of the blood vessels; frequently the reaction fails to occur, or else is paradoxical.

Similar effects are observed during the formation of conditioned vascular reflexes. Typically, at the first combined application following verbal instruction, in people of the strong balanced type, the vascular response is well shown.

It should be noted that differences in the lability of the cortical processes was not established by us for all the subjects. In all the 70 people investigated, the reversal of the conditioned reflexes occurred equally rapidly. It must be supposed that the mobility of the cortical processes in man is so high by comparison with animals that reversal as a test for mobility is too easy a task whatever type of nervous system the subject may possess. A more precise method is required to determine differences in lability.

Thus, vascular response may serve as a criterion by which to determine certain typological features

of higher nervous activity in man, and principally to distinguish between balanced and unbalanced types.

SUMMARY

A plethysmographic study was made of oriented vascular reactions, unconditioned vascular reflexes, and conditioned reflexes in 70 healthy individuals living in similar conditions. The vascular reactions enabled an assessment to be made of certain typological features of higher nervous activity, particularly of equanimity and inhibitory power.

LITERATURE CITED

1. S. P. Botkin, A Course on the Clinical Treatment of Internal Diseases, 1 [in Russian] (St. Petersburg, 1899).
2. K. G. Nikulin, Klinich. med. 30, 75 (1952).
3. A. T. Pshonik, The Cerebral Cortex and Receptor Function of the Organism [in Russian] (1952).
4. A. A. Rogov, Vascular Conditioned and Unconditioned Reflexes [in Russian] (1951).
5. I. I. Speranskii, Ter. arkhiv. 24, 23 (1952).
6. I. F. Suvorov, Analysis of Taste Reception in Man by the Method of Vascular Conditioned Reflexes [in Russian] (Dissertation) (Leningrad, 1949).
7. R. A. Fel'berbaum, (Dissertation) Analysis of Cutaneous Pressure Reception by the Method of Vascular Conditioned Reflexes (Dissertation) [in Russian] (Leningrad, 1950).
8. I. S. Tsitovich, Russki fiziol. zhur. 1, 113 (1918).
9. M. V. Chernorutskii and P. S. Lang-Belonogova, Klinich. med. 32, 3 (1954).

CARBON DIOXIDE SENSITIVITY OF THE RESPIRATORY CENTER IN CHRONIC HYPERCAPNIA

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The effect of carbon dioxide in exciting the respiratory center in subjects with chronic hypercapnia is of great interest in connection with the problem of to what extent the nervous center can adapt to the continuous influence of an adequate stimulus.

Scott [5] first investigated two subjects with chronic hypercapnia resulting from pulmonary emphysema; he determined the effect on pulmonary ventilation of breathing air containing an increased amount of CO_2. He found only a small increase in ventilation. A study of alveolar and pulmonary ventilation [4] when concentrations of 1, 3, and 5 % CO_2 were breathed by healthy subjects and by patients with pulmonary emphysema having an abnormal blood CO_2, showed that, normally, inhaling 5 % CO_2 increased ventilation by an average of 187 %, while in patients with a marked hypercapnia the increase was only 58 %. The authors throught that this small increase was due both to the increased alkali reserve and to a reduced sensitivity of the respiratory center to carbon dioxide.

The great difference in sensitivity of the respiratory center between healthy subjects and those with pulmonary emphysema was observed by Tenney [6], who thought that in addition to changes in the physicochemical constitution of the blood and to the mechanical interference with respiration, there is also some adaptation of the respiratory center to the action of carbon dioxide which develops in patients as a result of prolonged hypercapnia.

Two considerations have been advanced to explain this phenomenon.

The first inhalation of a gaseous mixture containing a certain concentration of CO_2 has a smaller excitatory effect on the respiratory center because of the change in the constitution of the blood (increased alkali reserve). However, this can scarcely be the reason for the reduced response from the respiratory center, because under the influence of the inspired CO_2, the partial pressure of the gas in the blood ought to increase to its previous level independent of any changes in the alkaline reserve, and its stimulant effect on the respiratory center should not be changed.

The second consideration is that under the influence of a prolonged hypercapnia, the sensitivity of the respiratory center to CO_2 is diminished. Shik, Kulik, and Shneiderovich [2] observed that the degree of chronic hypercapnia was inversely related to the increased ventilation in response to CO_2. Assuming that in prolonged hypercapnia the sensitivity of the respiratory center to CO_2 is reduced, they attempted to demonstrate this fact on animals which were kept for a considerable time in an atmosphere containing from 6-8 % carbon dioxide. However, when the sensitivity of the respiratory center of these animals to carbon dioxide was tested, it was found that it was not reduced and that the increased ventilation caused by the addition of CO_2 was no greater than it was before the subject had been exposed to it for a long time. The authors therefore concluded that the failure of patients to respond adequately by increased pulmonary ventilation to added CO_2 does not result from prolonged hypercapnia.

Therefore the problem of whether there is a change in excitability of the respiratory center to carbon dioxide (i.e., the question of whether there is or is not an adaptation) in patients with chronic hypercapnia still remains unsolved.

The present work represents an attempt to determine whether chronic hypercapnia does reduce the sensitivity of the respiratory center to CO_2.

METHOD

We used the method of electromyography of the respiratory muscles and made simultaneous recordings of the depth and frequency of respiration, as described previously [1]. For four minutes, the patient breathed into a Krogh spirometer from which the carbon dioxide absorbent had been removed; as a result the carbon dioxide gradually increased, finally reaching a level of 5-6 %. A study was made of 28 subjects suffering from pulmonary emphysema with chronic hypercapnia.

RESULTS

Analysis of the alveolar air and determination of the oxyhemoglobin in some of the patients showed that there was a marked hypoxemia and hypercapnia.

We used a concentration of CO_2 for the inspired air which in healthy human subjects caused an increased of pulmonary ventilation of 2-3 times or more, the increase being chiefly through increased depth of respiration.

In emphysematous patients, under these conditions the pulmonary ventilation increased considerably less, a result which agreed with published reports (Fig. 1). This effect was particularly marked for the depth of respiration. In the subjects investigated, the initial depth varied between 400 and 750 ml. After breathing CO_2, the depth increased to 600-1500 ml. An increase of two or more times compared with the normal value was found only in ten subjects; in 18, the increase was very much less, and in most of the patients the increase was not greater than 200-300 ml. The same thing was true of the total pulmonary ventilation, although in this case the difference between the healthy subjects and patients was less marked, because the increased ventilation in the patients occurred mainly through increase in the respiratory rate.

From the electromyograms and spirograms it was found that in spite of a small increase in the depth of respiration under the influence of carbon dioxide, the potentials in the respiratory muscles, which were abnormally large in the sick group even at rest, were further greatly increased when CO_2 was given, and reached a level considerably higher than that of the same muscles in healthy human subjects under the same conditions (Fig. 2). Therefore inspiring air enriched with carbon dioxide in hypercapniaic patients caused a considerable increase in excitation of the respiratory center, and it is impossible otherwise to explain the increased electrical activity of the respiratory muscles.

In all previous investigations, pulmonary ventilation has been taken as the index of activity of the respiratory center. However, under these conditions, when pulmonary ventilation is limited for other reasons, or because of pathological processes, the normal correspondence between the degree of excitation in the respiratory muscles and pulmonary ventilation is not maintained. In these cases

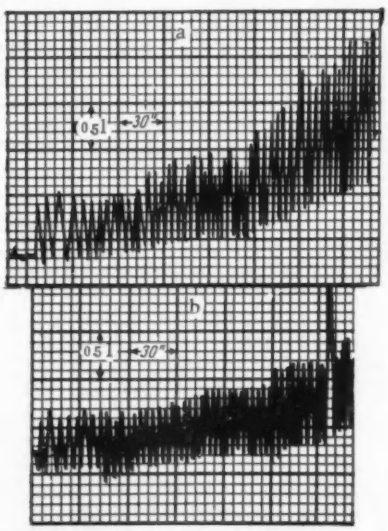

Fig. 1. Change in the pulmonary ventilation (spirograph curves) on breathing into a closed system in which the carbon dioxide concentration gradually increased. a) In a healthy subject; b) in a patient with pulmonary emphysema.

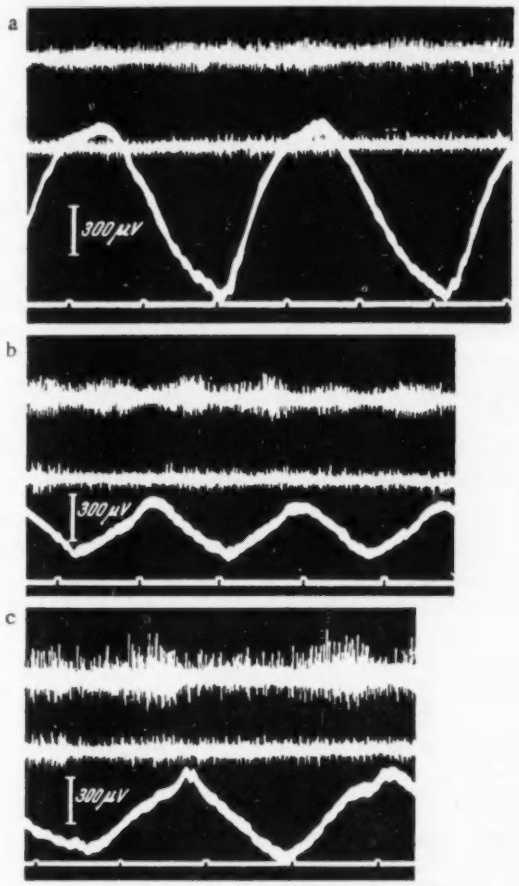

Fig. 2. Action potentials of the respiratory musculature. a) In a healthy human subject when breathing air containing 5 % CO_2; b) in a subject with pulmonary emphysema (second order respiratory insufficiency) when breathing normally; c) in the same subject, when breathing air containing 5 % CO_2. Curves from above downwards: electromyogram of intercostal muscles; electromyogram of the external oblique muscle of the abdomen; pneumogram; time marker (1 sec).

the electromyogram may give information about the flow of nervous impulses to the respiratory muscles, and from this information the condition of the respiratory center may be inferred.

The lack of correlation between the work of the respiratory muscles and the pulmonary ventilation rate in patients with pulmonary emphysema has previously been pointed out [3]. It was found that in them, the oxygen consumed by the respiratory muscles in breathing against the increased resistance rose considerably in response to quite small changes in the ventilation rate.

From our results it can be seen that the apparent reduction or absence of a respiratory response to carbon dioxide in cases of chronic hypercapnia is not due to loss of sensitivity of the respiratory center, as Cournand [4] and his co-workers supposed. When the carbon dioxide content of the inspired air is increased, the respiratory center is excited and causes a considerable increase in the flow of impulses to the respiratory muscles, causing an increased electrical activity in them. However, the increased muscular activity does not result in any great increase in the depth of respiration, because in a patient with pulmonary emphysema many factors impede ventilation; these include changes in the respiratory apparatus itself which causes the respiratory muscles to be ineffectual. In four out of the 18 patients in whom the added CO_2 caused only a small increase in ventilation, the electrical activity of the respiratory muscles also showed little change. In certain cases it may be that in addition to the reason given above, there is also a reduced nervous response to carbon dioxide.

Therefore, in chronic hypercapnia failure of respiration to respond to CO_2 is due mainly to pulmonary changes and not to adaptation of the respiratory center or of the chemoreceptors.

SUMMARY

By recording both the pulmonary ventilation and electrical activity of the respiratory muscles, the excitability of the respiratory center to carbon dioxide was investigated in patients with chronic hypercapnia due to emphysema of the lungs.

It was found that the electrical activity of the muscles responded sharply to CO_2, showing that the reduced respiratory response to CO_2 in chronic hypercapnia is due chiefly to pulmonary changes and not to adaptation of the respiratory center.

LITERATURE CITED

1. I. A. Morozova and L. L. Shik, Byull. Éksp. Biol. i Med. Ns. 5, 61 (1957).
2. L. L. Shik, A. M. Kulik,* and M. G. Shneiderovich, Oxygen Therapy and Oxygen Insufficiency [in Russian] (Kiev, 1952), p. 53.
3. E. J. Campbell, E. K. Westlake, and R. M. Cherniak, J. Appl. Physiol. 11, 303 (1957).
4. A. P. Fishman, P. Samet, and A. Cournand, Fed. Proc. 13, 44 (1954).
5. R. W. Scott, Arch. Intern. Med. 26, 544 (1920).
6. S. M. Tenney, J. Appl. Physiol. 6, 477 (1954).

* Original Russian pagination. See C.B. Translation.

GASEOUS CONTENT OF THE BLOOD IN THE UMBILICAL VESSELS IN INFANTS IN WHOM RESPIRATION DEVELOPS NORMALLY AT BIRTH

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The problem of the gradual change in gaseous content of the blood in the umbilical vessels which occurs at the time between normal delivery and the changeover to pulmonary respiration is important in many respects. Determination of the gaseous content of the blood, and particularly the oxygen content in the umbilical vein, gives information about the gaseous exchange occurring in the placenta before the child is delivered and while it maintains connection with the mother through the umbilicus. From the composition of the blood, and particularly from that in the umbilical vein, it is possible to determine the oxygen and carbon dioxide content at the time when pulmonary respiration starts, when it must be remembered that in normal births the umbilicus has not yet been tied off. The problem has already been solved for animals [1], but no corresponding studies in newborn infants are known to us. The results obtained by Arshavskii [1] have shown that the change in the composition of the blood gases, particularly in the umbilical arteries, reveals the rate of expansion of the pulmonary parenchyma after the first extrauterine respiratory movements. To determine the rate and the extent of the pulmonary expansion after birth, it was convenient to use the x-ray method which has been applied extensively by P. N. Serzhanin [2]. However, in addition to several practical difficulties and other undesirable complications associated with the application of this method to childbirth, as Arshavskii states, the first x-ray study cannot be made earlier than 5-6 min after the birth, i.e., at a time when in a normal birth a large amount of the pulmonary parenchyma has already expanded. Arshavskii's method is based on the following considerations: the two internal iliac or hypogastric arteries give rise to two umbilical arteries which carry venous blood to the placenta. As soon as the fetus changes over to pulmonary respiration, the blood in the umbilical arteries flowing in from the hypogastric arteries changes its gaseous composition and becomes arterial. The umbilical arteries do not close immediately; the rate of change of the gaseous composition of the blood in the umbilical arteries, i.e., the rate at which it becomes arterialized, is an indication of the rate of the transition to pulmonary gaseous exchange, and therefore of the rate of expansion of the lungs. Until now, a few authors [4-7] have studied the oxygen and carbon dioxide content in the umbilical vessels together in a single test.

In the present work we have aimed at determining changes in the gaseous composition of the blood in the umbilical arteries by withdrawing a series of blood samples from them.

As far as we know, no previous attempt has been made to use this method to determine both the composition of the blood and its changes in the umbilical arteries.

METHOD

In each infant, from 2 to 5 samples were withdrawn from the umbilical arteries. The first sample was taken 20-30 sec after birth, and the remainder at intervals of one or a few minutes. To prevent the infant from cooling, immediately after the birth it was placed in a bath filled with warm water at 37-38°. Care was taken not to compress the umbilical arteries with the hands when drawing blood. Blood samples of 2-3 cm^3, which was a sufficient quantity for the main and for the repeated (control) investigations, were withdrawn into a sterile syringe having a well-ground but freely mobile piston. The blood was kept under oil in a refrigerator for 1 to 6 hr, and the blood gases were determined using the manometric model of Van Slyke's apparatus.

Blood samples from the umbilical arteries were taken from the veins of 27 normal newborn infants delivered at term in the No. 7 Grauerman Maternity Home. The results obtained are shown graphically in Fig. 1.

RESULTS

Blood samples were collected from the umbilical vein at definite time intervals from 15 children, and in

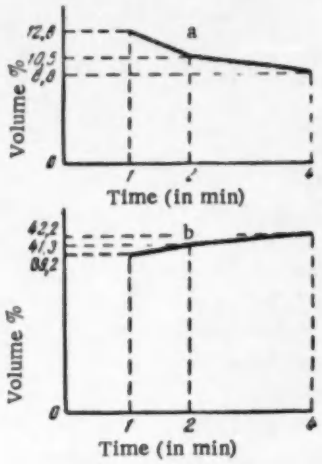

Fig. 1. Measurement of the percentage (a) oxygen and (b) carbon dioxide in the blood of the umbilical vein after birth.

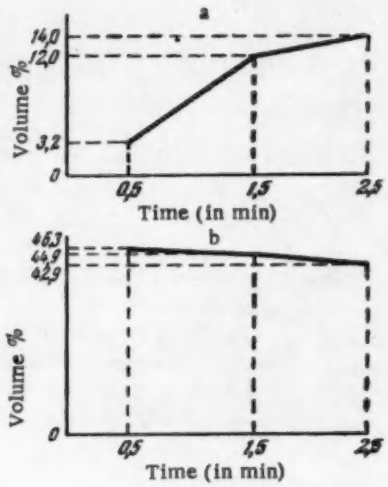

Fig. 2. Change in the percentage of (a) oxygen and (b) carbon dioxide in the blood of the umbilical artery after birth.

12, samples were collected from the umbilical artery.

According to Barcroft [3], in normal placental gaseous exchange, the blood in the umbilical vein is 95 % saturated with oxygen. In view of the high oxygen capacity of the fetus at term, the oxygen content of the blood of the umbilical vein would be expected to lie between 19 and 20 %.

Of the 15 infants which we examined, high values of 18.8 and 17.9 % oxygen in the umbilical vein were found only in two cases. In two others, the value lay between 16.4 and 15.7 % in the first minute. In the remainder, during this time it varied from 14.3 to 9.1 %. In every case, the amount of carbon dioxide during this period varied from 37 to 45 %, and depended on the concentration of oxygen in the umbilical vein.

In every case, the newborn infant drew the first extrauterine breath, and accordingly cried, within the first minute. Our results did not allow us to determine exactly what was the oxygen content of the blood of the umbilical vein at the moment that the first breath was taken, because this event occurred during the first minute when fluctuations took place as described above. It must be emphasized that in two cases breathing started when the oxygen content in the umbilical vein was 18.8 and 17.9 %. Blood gas analyses made during the next 3-4 min showed a progressive reduction in the amount of oxygen, and, correspondingly, some increase in CO_2. By the end of the 3-4th min, the oxygen had been reduced to 10-8 %, and in one case at the end of the fourth minute it had reached 6.1 %. Figure 1 shows a typical case of the change in oxygen and carbon dioxide contents in the blood of the umbilical vein after birth.

The progressive reduction in the oxygen content of the blood in the umbilical vein, which we found to begin after the second, and to increase rapidly after the third, minute, does not result from a cessation of placental gaseous exchange caused by separation of the placenta. The oxygen content of the blood in the umbilical vein recorded during the first minute is a reliable index of the completeness of the connection between the placenta and the uterine mucosa.

In normal newborn infants, pulsation of blood in the umbilical arteries continues during this period. On this account, the results which we obtained indicate that there is considerable variation in the extent to which the placenta has separated at birth. Thus, in cases in which the oxygen content was 18 %, it can be deduced that the birth occurred without any marked separation of the placenta. In cases where the oxygen content in the umbilical vein was 6 or 8 %, there must have been considerably more separation.

In most cases, the oxygen content in the umbilical artery varied from 3 to 6 % in the first minute, and the carbon dioxide concentration, from 45 to 48 %. In subsequent tests there was a progressive increase in blood oxygen and a corresponding decrease in carbon dioxide. Figure 2 illustrates a typical case of these blood gas changes after birth. In most cases, by the end of the third minute the blood oxygen in the umbilical artery was 12-14 %. Taking into account the oxygen capacity of the blood of the newborn child, it can be seen that the lungs are more or less fully expanded when the oxygen concentration in the umbilical artery reaches 19-20 %. It would be difficult to obtain samples of blood from

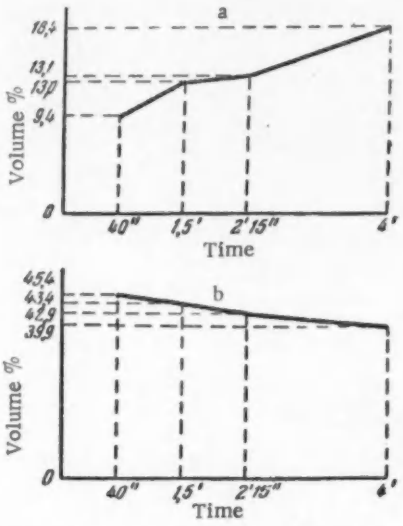

Fig. 3. Changes in the percentage oxygen content (a) and carbon dioxide (b) in the blood of the umbilical artery after birth. In this case the lungs were fully expanded at the 4th minute after birth.

these arteries 3 min after birth, not only because the artery ceases to pulsate, but because it begins to empty. In one case, blood taken at the end of the fourth minute had an oxygen content of 18.4 % (Fig. 3), indicating that the lungs were quite fully expanded. It would not be legitimate to infer that by the 4th minute, when respiratory movements are completely established, the lungs are more or less completely expanded. To establish this fact, a simultaneous x-ray examination is required. In the 27 newborn infants, the duration of the umbilical pulse was measured. In 4 it continued for 1 min, in 8 for 2 min, in 12 for 3 min, and in 3 for 4-5 min. When the pulsation in the cord ceases, the umbilical arteries empty rapidly. When this occurs it becomes increasingly difficult to withdraw blood from them. The initial closure of the umbilical arteries and their subsequent gradual emptying is probably caused by the progressive fall in the oxygen content in the umbilical vein which has been mentioned above, and which occurs approximately one minute after birth. For the same reason, the amount of blood passing into the placental capillaries is reduced. Because the amount of blood involved in gaseous exchange with the maternal blood through the placental capillaries becomes reduced, there is naturally a progressive reduction in the oxygen content of the blood in the umbilical vein, which in this case cannot be ascribed to separation of the placenta.

Thus by measuring the oxygen and carbon dioxide contents of umbilical arterial blood in normal newborn infants by taking successive blood samples, it was shown that even 3-4 min after birththere is a marked increase in the oxygen content indicating a rapid expansion of the lungs and a transition to pulmonary gaseous exchange as the first extrauterine respiratory movements take place.

The variation of from 6 to 18.8 % in the oxygen content of the blood of the umbilical vein during the first minute indicates that there may be different degrees of separation of the placenta at birth.

SUMMARY

Measurements were made of the oxygen and carbon dioxide concentrations in successive samples of blood taken from the umbilical arteries in normal newborn infants. The oxygen content was found to rise considerably as early as the 3-4th min after birth. This points to a rapid pulmonary expansion and a changeover to gaseous exchange in normal infants performing normal initial respiratory movements. The normal range of blood oxygen concentrations within the first minute is from 6-18.8 %. This shows that in normal births labor may terminate with various degrees of placental detachment; evidently when the oxygen content was 18 %, labor terminated before detachment, while when it was only 6-8 %, the infant was delivered after the placenta had separated to a greater extent.

LITERATURE CITED

1. I. A. Arshavskii and N. E. Ozeretskovskaya, Byull. Éksp. Biol. i Med. 15, 42 (1943).
2. P. N. Serzhanin, Expansion of Pulmonary Tissue in Newborn Infants as determined by X-Ray Investigations Dissertation [in Russian] (Moscow, 1958).
3. J. Barcroft, Researches on Prenatal Life (Oxford, 1946).
4. M. Bidone, Ann. Ostet. Gynec. 53, 197 (1931).
5. N. Eastman, Bull. Johns Hopkins Hosp. 50, 39 (1932).
6. G. Haselhorst and K. Stromberger, Z. Geburtsh. und Gynäk. 98, 49 (1930).
7. M. Noguchi, Japan. J. Obst. and Gyn. 20, 218 (1937).

BREATHING A HELIUM-OXYGEN MIXTURE WHEN PULMONARY GASEOUS EXCHANGE IS IMPAIRED

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A great deal of work has been done to determine the usefulness of breathing a mixture of helium and oxygen in bronchial asthma, pulmonary emphysema, bronchitis, tracheitis, and pulmonary tuberculosis [2-8].

It has been shown that a favorable effect is obtained.

The result is explained as being due to the fact that the density of the mixture is very much less than that of atmospheric air or pure oxygen, so that less effort is required to overcome the resistance of the current of air at inspiration and expiration.

In our laboratory [1] it was shown long ago in human subjects performing muscular work that when additional respiratory resistance had been artificially introduced, respiratory efficiency was markedly reduced, with the result that there was a reduction in the oxygen saturation of arterial blood.

This led us to use a mixture of helium and oxygen under these conditions.

METHOD

The experiments were carried out on 8 women and 3 men aged from 25 to 30 years.

The subjects breathed a gaseous mixture consisting of 20 % oxygen and 80 % helium, and extra resistance was introduced both while they were at rest or performing muscular work.

The additional resistance was introduced by using water valves, and a water column height of 100 mm. The resistance was effective both at inspiration and expiration. Records were made of pulmonary ventilation, arterial blood oxygen saturation, and intrapulmonary pressure at both inhalation and exhalation (using a water manometer).

At rest, breathing through the added resistance was continued for 12-16 min, and when working for 16 min.

Both at rest and when working, every four minutes, the helium-oxygen mixture was replaced by atmospheric air.

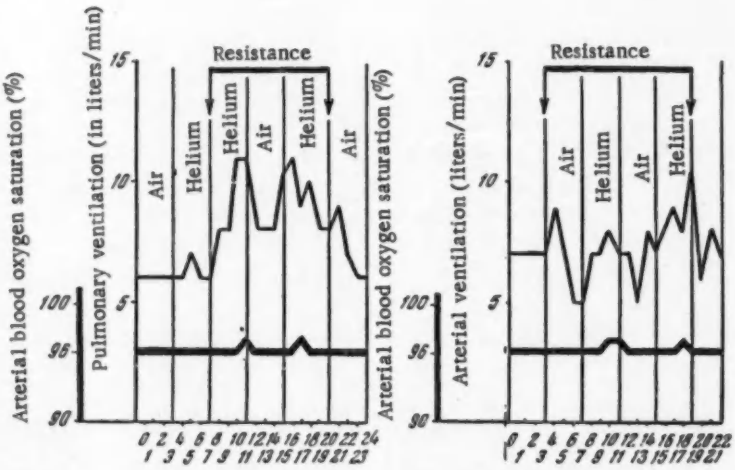

Fig. 1. Change in pulmonary ventilation and arterial blood oxygen saturation when breathing a helium-oxygen mixture through an increased resistance at rest.

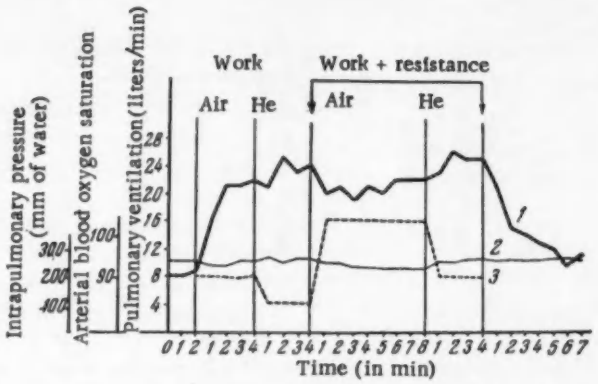

Fig. 2. Change in pulmonary ventilation (1) and arterial blood oxygen saturation (2) on breathing a helium-oxygen mixture during muscular work with and without an added respiratory resistance.

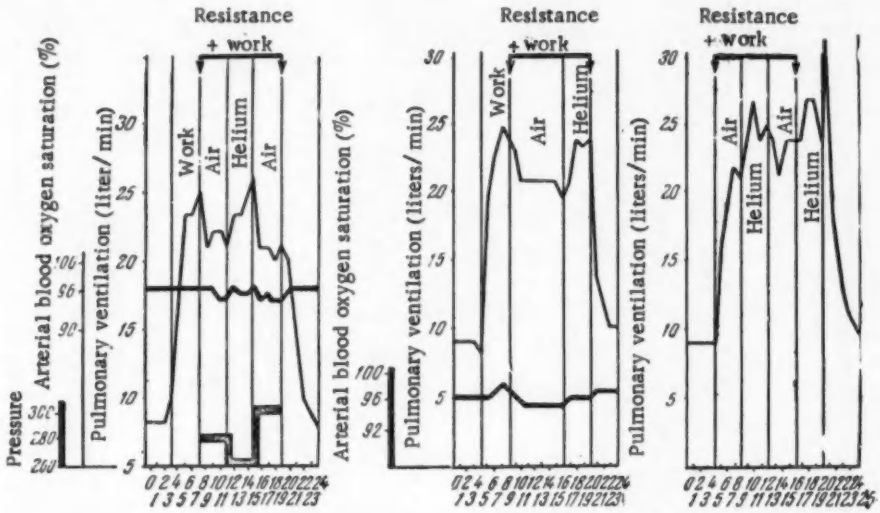

Fig. 3. Change in pulmonary ventilation and arterial blood oxygen saturation when breathing a helium-oxygen mixture during work with added respiratory resistance.

It was found that at rest, and when breathing normally, the mixture caused no noticeable respiratory changes.

When breathing against a resistance, both when inhaling and exhaling, the effectiveness of the breathing was found to depend to a great extent on whether the subject was breathing atmospheric air or the helium - oxygen mixture. When breathing the mixture, ventilation increased by 1-4 liters/min, it caused some increase in the oxygen saturation of the blood, and intrapulmonary pressure was reduced to a value below that obtaining when atmospheric air was breathed (Fig. 1).

Similar changes in respiratory movements were observed when the subject breathed the helium-oxygen mixture while performing work on a bicycle ergometer. The pulmonary ventilation was then 3-5 liters/min greater than when the same work was performed while atmospheric air was breathed, while, on the other hand, the pulmonary pressure was reduced by 20-30 mm of water.

Breathing the helium and oxygen mixture proved particularly effective when performing work and breathing through an added resistance. Usually, pulmonary ventilation was reduced when air was breathed, but under these conditions it began to increase as soon as the helium-oxygen mixture was given. The ventilation increased by 3-6 liters/min. At the same time there was an increased arterial blood oxygen saturation and a reduced intrapulmonary pressure (Fig. 2 and 3).

The results showed, therefore, that when breathing was made more difficult by adding the extra resistance,

the helium-oxygen mixture facilitated respiration by virtue of its physical properties.

This result apparently explains the useful effect obtained by giving a helium-oxygen mixture in diseases of the respiratory system, when breathing is impaired as a result of the pathological changes.

SUMMARY

Inhalation of a mixture containing 80 % helium and 20 % oxygen when respiratory resistance was artificially increased caused the following changes: pulmonary ventilation increased, there was a greater arterial blood oxygen saturation, and intrapulmonary pressure was reduced. The respiratory changes were more pronounced during exercise than at rest.

LITERATURE CITED

1. A. M. Kulik, The Physiology and Pathology of Respiration, Hypoxia, and Oxygen Therapy [in Russian] (Kiev, 1958) p. 211.
2. A. L. Barach, Proc. Soc. Exper. Biol. and Med. 32, 462 (1934).
3. A. L. Barach, J. A. M. A. 107, 1273 (1936).
4. D. Bates and R. Christie, Clin. Sci. 9, 17 (1950).
5. W. A. Briscoe, Clin. Sci. 10, 37 (1951).
6. W. A. Briscoe, Clin. Sci. 11, 45 (1952).
7. U. H. Eversole, J. A. M. A. 110, 878 (1938).
8. E. Ricen, U.S. Nav. Med. Bull. 40, 853 (1942).

REFLEX INFLUENCE OF THE PRESSURE RECEPTORS OF THE STOMACH ON THE RENAL CIRCULATION IN NORMAL CONDITIONS AND EXPERIMENTAL RENAL HYPERTENSION

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In recent years the mechanism of regulation of the regional circulation of the blood has occupied the attention of researchers on an increasing scale. Work recently published has shown that reflex changes in the level of the arterial blood pressure, of different origin, may be based upon changes of vascular tone in various regions of the body, revealed in different ways and in different combinations. A. M. Blinova, G. N. Aronova, and K. E. Serebryanik [1] observed dissimilar changes in the tone of the vessels of the kidneys, intestine and limbs in the course of pressor reactions as a result of stimulation of the thalamic region, compression of both common carotid arteries and the injection of promethine. From specially carried out investigations, these authors conclude that the distinctive reactions of different vascular fields in the mechanism of the pressor reactions under investigation are due to changes in the functional state of the vasomotor center. V. M. Khayutin [8] found that the reactions of the renal vessels are dependent on the character of the stimulation; they are more pronounced in response to stimulation from the interoceptors of the large intestine, and decrease in the order : urinary bladder, tibial nerve, carotid sinus.

By investigating the electrical activity of the preganglionic branches of the sympathetic nervous system (cervical and splanchnic nerves), N. K. Saradzhev [7] concludes that the spread of excitation from the vasomotor center during the pressor reactions under test to the mechanoreceptors of the urinary bladder and carotid sinuses is not the same. T. S. Lagutina [6], also by electrophysiological methods, investigated the relationship between the reflexes proper and the stretch reflexes developing during increasing intensity of stimulation of the mechanoreceptors of the urinary bladder.

The object of the present investigation was to study the changes in the renal circulation under the influence of "stretch" reflexes from the pressure receptors of the stomach, and to compare these with the results previously obtained on the character of the reactions of the renal vessels to reflex influences from other interoceptive fields [2]. It was also very interesting to discover whether there is any difference in their reactions when the vascular tone is pathologically changed during experimental renal hypertension. According to V. N. Chernigovskii, in the case of "stretch" reflexes, other physiological systems besides that with which the particular interoceptive zone is connected are involved in the reaction.

The present work is the continuation of investigations conducted over a period of several years in our laboratory on the role of the renal pressor factor in the regulation of the level of the blood pressure in normal conditions and in experimental hypertension.

EXPERIMENTAL METHOD

The animals were investigated on an empty stomach. In the first place, they were given a high water intake: rabbits received 40 cm^3 water per kg body weight by gastric tube, dogs, 600-800 cm^3 milk, diluted with water. Anesthesia was by intravenous injection of nembutal (40 mg/kg body weight). The renal circulation was investigated by Rein's thermoelectric method. The thermoelements were applied transperitoneally to the renal artery (or one of its branches) and to the renal vein to check the inflow and outflow of blood from the kidneys. The operation wound was carefully sutured in layers. At the same time, the changes in the level of the blood pressure in the femoral artery were recorded on a mirror galvanometer by means of a photocell. The pressure receptors of the stomach were stimulated by distension of a thin rubber balloon, introduced into the stomach by means of a tube (50-100 cm^3 of air in the case of rabbits, and 200-600 cm^3 of air in dogs). At the beginning of the experiment less intensive stimuli were usually used, and then a change was made to more intensive. Stimulation lasted for 2-2.5 min. The intervals between the periods of stimulation were not less than 15-20 min. Experimental renal hypertension was induced by the routine method used in our laboratory. The animals were investigated in the 2nd-4th month of development of hypertension. In all, 37 experiments

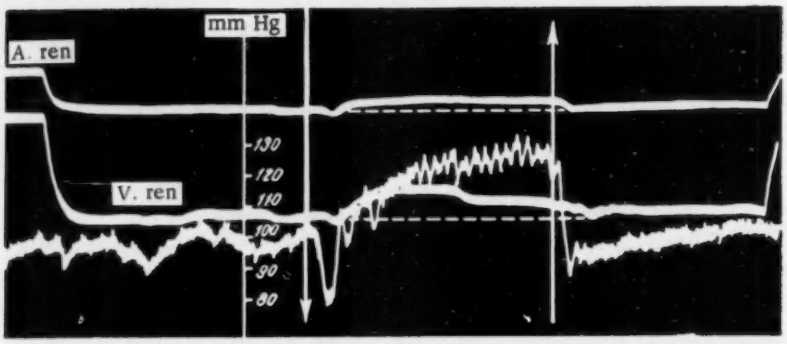

Fig. 1. Increase in the renal blood flow simultaneously with an increase in the blood pressure during inflation of a rubber balloon in the stomach with 100 cm^3 of air (experiment on a rabbit). Significance of the curves (from above, down): blood flow in the renal artery , the same in the renal vein, blood pressure in the femoral artery; ↓ ↑) markers of the beginning and end of stimulation.

were performed; 19 of these were carried out on normal animals (13 rabbits and 6 dogs) and 18 on animals with hypertension (10 rabbits and 8 dogs). In the experiments on animals with hypertension, the inflow of blood to the kidneys was investigated in the pulsating segment of the renal artery distal to the clamp (in experiments on dogs the thermoelement was usually applied to one of the branches of the renal artery).

EXPERIMENTAL RESULTS

Comparison of the reactions of the renal vessels to stimulation from the various interoceptive areas in normal animals shows that their reaction to stimulation from the interoceptors of the internal organs varies much more than that to pressor influences from the pressure receptors of the carotid sinuses [2]. In a given case this is shown by the fact that, along with passive changes in the renal blood flow, active changes are also found in the tone of the renal vessels. The passive changes in the renal blood flow are expressed as changes in the blood flow in the renal artery and vein, occurring at the same time and in the same direction as the level of the blood pressure. By way of example of the passive influence of the raised blood pressure, under the influence of stimulation of the pressure receptors of the stomach, on the renal circulation, we give the results of one such experiment in Fig. 1. Increase in the blood pressure by inflation of the balloon by introduction of 100 cm^3 of air was accompanied in this experiment by a simultaneous increase in the blood flow in the renal vessels (experiment on a rabbit).

Active changes in the tone of the renal vessels—their dilation or constriction—may be judged by changes in different directions in the blood flow in the renal vessels and the level of the blood pressure [11-14 and others]. With dilatation of the renal vessels there is a fall in the blood flow in the renal vein, whereas the blood flow in the artery remains at the same level or may even be increased. The impression was created that, at a given moment, the inflow of blood exceeded the outflow, which was naturally only possible by an increase in the capacity of the blood vessels. Constriction of the renal vessels was demonstrated by the converse changes, namely, a fall in the blood flow in the renal artery and an unchanged, or even increased blood flow, especially at the beginning of stimulation, in the renal vein.

Of the 19 experiments carried out on normal animals in 10 (8 experiments on rabbits and 2 on dogs) the renal circulation was either unchanged or changed in a passive manner; in the remaining nine experiments an active reaction of the renal vessels was observed, and in particular in five experiments (on four rabbits and one dog), in the form of dilatation, and in four (one experiment on a rabbit and three on dogs) in the form of constriction of the renal vessels. In Fig. 2 are shown the results of one of the experiments, in which active changes in the tone of the renal vessels were observed in response to reflex influences from the pressure receptors of the stomach, in the form of dilatation.

The increase in the blood pressure as a result of inflation of the balloon in the stomach with 100 cm^3 of air was accompanied by a well-marked increase in the blood flow in the renal artery and a fall in the blood flow in the renal vein: evidence of dilatation of the vessels within the kidney. The changes described above in the blood flow in the renal artery and vein, reflecting active changes in the tone of the vessels within the kidney , may be extremely variously manifested; it may be gathered from this how different in their course the reactions of the vessels may be.

When stimulation of different intensities was used in the same experiment, the more intensive stimulation was accompanied usually by the more pronounced changes in the renal circulation; in some cases an active reaction of the vessels was observed.

The development of experimental renal hypertension was associated with the appearance of reactions of varying

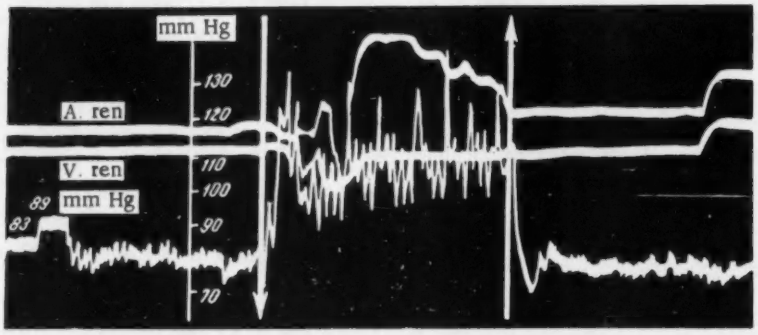

Fig. 2. Dilation of the renal vessels during inflation of a balloon in the stomach by introduction of 100 cm³ of air (experiment on a rabbit). Legend as in Fig. 1.

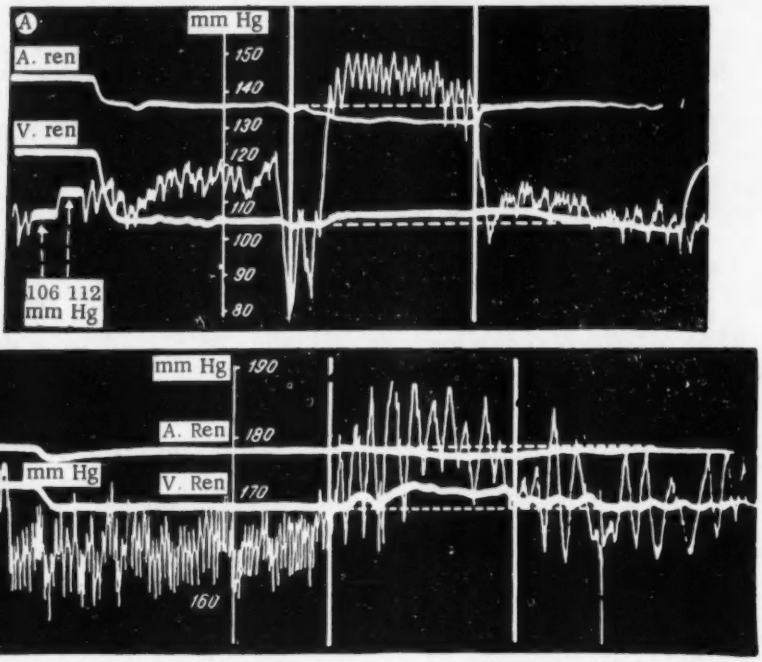

Fig. 3. Spasm of the renal vessels in response to reflex influences from the stomach in the presence of experimental renal hypertension. A) Experiment on a rabbit (inflation of the balloon in the stomach with 100 cm³ of air); B) experiment on a dog (inflation of the balloon in the stomach with 200 cm³ of air). Legend as in Fig. 1.

character in the renal vessels in response to reflex influences from the stomach. In contrast to normal animals, those with hypertension showed a clear tendency toward predominantly active reaction of the renal vessels in response to these influences in the form of spasm. When hypertension was present, this type of reaction in response to different forms of stimulation became so predominant that it was even impossible to detect any difference in the reaction of the renal vessels to stimulation from the stomach and from the carotid sinuses. Of 18 experiments performed on animals with experimental renal hypertension, spasm of the renal vessels in response to distension of the stomach was observed in 16 (9 on rabbits and 7 on dogs). In the remaining experiments dilatation of the renal vessels was found (one experiment on a rabbit and one on a dog).

Figure 3 shows the results of two experiments on animals with hypertension. We may see that stimulation of the pressure receptors of the stomach led to a fall in the blood flow in the renal artery and an increase in the blood flow in the renal vein, thus demonstrating constriction of the vessels within the kidney. At the end of stimulation the tone of the renal vessels returned to its original state, as shown by the restoration of the inflow of blood to and outflow from the kidneys.

It should be mentioned that the tendency toward spasm of the renal vessels in response to reflex influences

from the stomach was especially pronounced when the stimulation was very intensive. This distinctive character of the reaction of the renal vessels shows that, during hypertension, the renal pressor factor may participate in the mechanism of the interoceptive pressor reflexes, possibly acting as one of the humoral factors responsible for the degree and duration of these reflexes. It is brought into play reflexly, as the result of appropriate interoceptive influences.

Investigations carried out in recent years into the etiopathogenesis of the hypertensive state, both experimental and clinical, in the light of the views of Soviet scientists on the neurogenic nature of hypertension, have shown that the initial stages of hypertension are characterized by a high level of excitation of the higher divisions of the central nervous system and of the central apparatus regulating the circulation of the blood (G. F. Lang, A. L. Myasnikov, A. K. Dolin, S. D. Kaminskii, and V. I. Savchuk, N. N. Gorev, and M. I. Gurevich, and others). Changes in the functional state of the central nervous system with the appareance of increased excitation of the vasomotor apparatus are also clearly seen in animals with experimental renal hypertension [3-5, 10].

The difference observed in the present investigation between the reactions of the renal vessels in animals with hypertension and those in normal animals was evidently due to changes in the functional state of the central apparatus of regulation of the circulation of the blood.

SUMMARY

In normal animals, variations of reactions of the renal vessels to the reflex effect from the stomach are greater than their reactions in response to the effects from the carotid sinuses. In about 50 % of the experiments, the changes of the renal circulation were characterized by active changes of the renal vessel tone in the form of dilatation and constriction. Development of experimental renal hypertension is associated with the reaction of the renal vessels (mainly spastic in character) to the pressor effects from the stomach. Consequently, reflex effects from the stomach may occasion the functional inclusion of the renal pressor factor in the mechanism of pressor reactions in hypertension. The development of experimental renal hypertension is characterized by the increased excitability of the central circulatory control apparatuses. The distinction between the reactions of the renal vessels in animals with hypertension and those in normal animals may be explained by the difference in the intial functional condition of the vasomotor centers, against the background of which the stimulation is effected.

LITERATURE CITED

1. A. M. Blinova, G. N. Aronova, and K. E. Serebryanik, The Nervous Control of the Circulation of the Blood and of Respiration [in Russian] (Moscow, 1952), p. 211.
2. A. I. Vishatina, Fiziol. Zhur. Ukrain. 5, 75 (1959).
3. N. N. Gorev, Hypertension Studies [in Russian] (Kiev, 1959).
4. M. I. Gurevich, Fiziol. Zhur. Ukrain. 1, 62 (1955).
5. M. A. Kondratovich, Voprosy Fiziol. No. 7, 105 (1954).
6. T. S. Lagutina, Byull. Eksp. Biol. Med. No. 1, 3 (1959).*
7. N. K. Saradzhev, Fiziol. Zhur. SSSR 45, 65 (1959).
8. V. M. Khayutin, Byull. Éksp. Biol. Med. No. 10, 18 (1958).*
9. L. P. Cherkasskii, Voprosy Fiziol. No. 10, 107 (1954).
10. V. N. Chernigovskii, Zhur. Vysshei Nerv. Deyatel. 6, 53 (1956).
11. P. Eckardt, Arch. ges. Physiol. 236, 361 (1935).
12. T. Gotsev, Arch. ges. Physiol. 236, 220 (1935).
13. H. Rein, R. Liebermeister, and D. Schneider, Klim. Wschr. 11, 1636 (1932).
14. H. Schweiegk, and S. Pfluger, Arch. ges. Physiol. 236, 206 (1935).

* Original Russian pagination. See C.B. Translation.

CHANGES IN HEMODYNAMIC INDICES OF DOGS WITH EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS

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We have established that considerable hypercholesterolemia and weight changes are observed in young male dogs receiving by simultaneous enteral administration a solution of cholesterol in sunflower oil and 6-methylthiouracil in powder form for a period of 120 days. No persistent increase in the arterial pressure was observed under these conditions.

At morphological examination of the dogs, sacrificed 150 days after the beginning of feeding, the most pronounced changes were found in the coronary arteries of the heart and in the arteries at the base of the brain, i.e., in those parts of the cardiovascular system which are most commonly and most severely affected by arteriosclerosis in man.

Because of this resemblance, in the present research we made a dynamic study of the functional changes in the circulation of the blood during experimental cholesterol atherosclerosis in dogs.

EXPERIMENTAL METHOD

We used electrocardiography and the method of ballistocardiography, which enables the state of the contractile function of the myocardium to be assessed and is being used in experimental and clinical practice on an increasingly wide scale. An essential condition for the recording of the ballistocardiogram (BCG) is the provision of the most complete possible muscular rest of the animal. In acute experiments this difficulty is easily overcome by anesthesia [4], but in chronic experiments in which large numbers of repeated observations have to be made, the use of anesthesia is for obvious reasons undesirable.

The technique which we have devised [8] enables the BCG to be recorded by a direct method in chronic experiments on dogs without the use of anesthesia and fixation of the animal. Of the 12 dogs included in the experiment, the BCG was recorded regularly in ten. Two dogs were so excitable that, in spite of prolonged training, it was impossible to develop a pose reflex in them and to record the BCG and, moreover, when these animals were subsequently fed with cholesterol and 6-methylthiouracil, epileptic fits were observed.

Besides the BCG of velocity and the ECG, recorded in three standard leads by means of subcutaneous needle electrodes, determinations were also made of the velocity of spread of the pulse wave, which is known from clinical findings [2] to be increased in atherosclerosis. The pulse waves were recorded by means of two piezoelectric pickups, one of which was fixed to the left common carotid artery, exteriorized in a skin flap by operation, and the other was applied to the proximal segment of the left femoral artery. The distance between the pickups was determined as follows. The distance from the sternal notch to the carotid artery, from the carotid artery to the umbilicus and from the umbilicus to the point of registration of the pulse in the femoral artery was measured in the dog. We added the values of these segments and subtracted from the total obtained twice the distance from the sternal notch to the carotid artery, since the blood flow in the ascending aorta and the carotid artery is directed in an opposite manner to that in the femoral artery, and the distance from the sternum to the carotid artery practically corresponds to the length of the ascending portion of the aorta and also to the distance from the aorta to the carotid artery. Measurements on cadavers have shown that the distance, calculated in this way, from the heart to the femoral artery differs from the true distance by only 1-3 cm, which is negligible when the velocity of spread of the pulse wave is high [2].

The velocity of spread of the pulse wave was determined by simple arithmetical calculation from the time of delay in the appearance of the femoral pulse after the pulse in the carotid artery.

These indices were recorded at the same time as a tracing of the respiration, which is needed for interpretation of the BCG as well as for giving an idea of the animal's general condition. For recording the respiration, we used a piezoelectric pickup, fixed to the animal's chest wall.

Bearing in mind that moderate physical effort (running on a treadmill for 5 min at a velocity of movement of the belt of 6.5 km/hr) places increased demands on the heart, we considered that this would enable us to detect latent signs of failure of the coronary circulation, and

to give a better picture of the obvious signs. We therefore recorded all the indices (BCG, ECG, pulse waves and external respiration) before and after physical effort. The pickups and electrodes were connected by means of screened leads to the input of the amplifier of a 4-channel ink-recording apparatus for functional diagnosis, type 4-PFD-7. The calibration signal was of 500 μv. The pulse waves were recorded on paper moving at the rate of 6 cm/sec, and the ECG and BCG on paper moving at the rate of 3 cm/sec.

EXPERIMENTAL RESULTS

The experiments on the dogs were carried out in the morning, before the animals were fed, in standard conditions and in the prone position; before the animals were fed with cholesterol and 6-methylthiouracil they were kept under preliminary observation for 1½ -3 months. The heart rate of the dogs under these circumstances was 70-120/min, and slight respiratory arrhythmia and moderate tachycardia were observed after physical effort.

In the ECG at this period, the T wave in three dogs was positive and in one it was negative in all leads; in four dogs it was negative on lead I only; in one dog the ST interval in lead I was below the isoelectric line.

In the control group, consisting of two dogs, which received 6-methylthiouracil for 90 days, the T wave was positive in the three standard leads, and apart from a slight fall in the voltage in lead I, we found no changes in the ECG at this period. In the animals of the experimental group, tachycardia appeared 2-2½ months after the beginning of feeding, and was especially marked after physical effort (in some dogs the quickening of the heart rate reached 100%). The voltage of the ventricular complex QRS was decreased, especially in lead I. Changes and inversion of the T wave developed in different leads (most often in lead III), displacement of the ST interval above and below the isoelectric line was observed, especially in leads I and III (Fig. 1). In the course of feeding of the dogs, these phenomena became more pronounced and the T wave in the ECG of some dogs became hardly perceptible, whereas in others it increased in size and equalled in magnitude the height of the R wave. In some dogs the T wave became rounded in shape and the P wave became smaller in size and smoother in shape. In four dogs an extrasystolic arrhythmia appeared, at first coming on before effort and disappearing afterwards; later, however, on the contrary, the extrasystoles appeared most often after physical effort. This phenomenon is evidently explained by the different situation and degree of the developing atherosclerotic changes in the coronary vessels. At first, with slight changes in the arteries of the heart, these vessels were dilated under the influence of physical effort and the blood supply to the myocardium improved. Later, when more advanced changes had developed in the arteries, the vessels were incapable of adequately nourishing the heart to cope with the increased demands made on the cardiac muscle by physical effort, and the conducting system of the heart could not ensure the regular rhythm of the contractions. At this period moderate physical effort became too much for the animals, and they fell on the treadmill after 2-3 min, running only 300-350 m at a velocity of 6.5 km/hr. The ECG showed signs of atrioventricular block, and isolated P waves appeared on a background of the extrasystolic arrhythmia. The ECG changes, especially after physical effort, revealed abnormalities in the dogs similar to those observed in patients with coronary insufficiency [1] and also in dogs with experimental cholesterol atherosclerosis according to the findings of other authors [5]. The opinion is held that the ECG of dogs

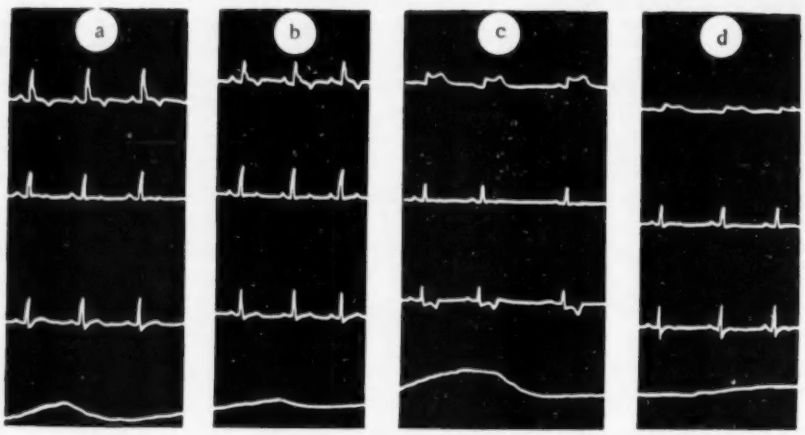

Fig. 1. Electrocardiogram of dog no. 7 in three standard leads. Significance of the curves (from above, down): I, II, III) ECG leads; respiration. a) Before feeding on cholesterol and 6-methylthiouracil, at rest; b) after effort; c) 2 weeks after cessation of feeding, at rest; d) after effort.

with experimental atherosclerosis is unchanged [9]. Disturbance of the circulation in the myocardium led to changes in the relationships of the ballistic forces, and was investigated by repeated recording of the BCG. The ballistographic complexes on the initial tracings had a well-marked constancy of shape and amplitude in the phase of expiration, and in inspiration, in consequence of respiratory arrhythmia, were nearly always superimposed on each other. In expiration the H wave was smaller than I, J, and K; the I wave was smaller than J and K; J was smaller than or equal to K. Well-marked atrial and diastolic waves were observed. After physical effort the complexes were unaltered provided that the dogs did not develop severe dyspnea and tachycardia. As feeding of the animals with cholesterol and 6-methylthiouracil continued, their BCG underwent definite changes. The general amplitude of the complexes fell significantly and all the waves became rounded and smoothed. Starting after 1½-2 months, the H wave decreased and then increased in size, and the "early" M waves on the BCG became clearly visible, which was a sign of weakening of the force of contraction of the heart. The I wave was greatly diminished, which also was a sign of the weakening of the contractile function of the myocardium in the phase of expulsion. The J wave was usually split up after physical effort, which was a sign of asynchronism of the expulsion of blood from the right and left ventricles. The K wave was deepened, which also suggested weakening of the expulsion of blood into the aorta. The atrial waves were decreased in size and the diastolic waves were reduced in some dogs and enlarged in others. The character of the changes in the BCG thus demonstrated weakening of the contractile function of the myocardium, which is observed in human subjects in coronary insufficiency [3, 6]. In some dogs the changes in the ECG and BCG developed along parallel lines; in the majority of the animals the changes in the BCG appeared earlier than those in the ECG; the BCG of the control dogs was unchanged.

The velocity of spread of the pulse wave in the period of preliminary observation of the dogs was from 7.05 to 10.8 m/sec, and in the course of the experiment it sometimes (from experiment to experiment) was changed by 10-15%, which could be considered to be physiological variations in the tone of the vessels [2]. The velocity of spread of the pulse wave is almost entirely determined by the degree of elasticity of the aortic compression chamber, and to a lesser degree by the force and rapidity of the expulsion of blood into it from the left ventricle [7]. According to the BCG, findings, the contractile function of the myocardium in the experimental dogs was considerably weakened.

On the other hand, the elasticity of the aorta and the main arteries was unchanged, which is understandable because subsequent morphological investigations of the aorta of the experimental dogs revealed no significant changes in their structure apart from a varying degree of lipoidosis. The increase in the velocity of spread of the pulse wave, frequently observed in clinical practice, must evidently be attributed to the gross morphological changes in the aortic wall with the proliferation of connective tissue, arising at a later stage of atherosclerosis, i.e., when signs of sclerosis are well marked.

The importance attached by clinicians to the velocity of spread of the pulse wave in the diagnosis of the early stages of atherosclerosis [2] is not, therefore, supported by our experimental findings.

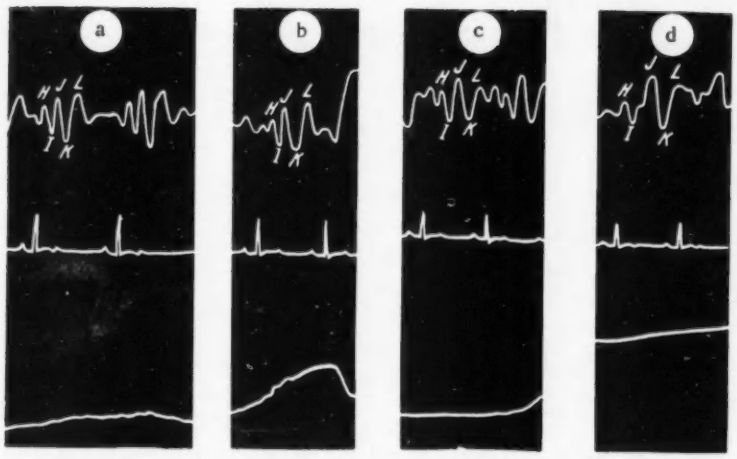

Fig. 2. Direct ballistocardiogram of velocity in dog no. 7. Significance of the curves (from above down): BCG; ECG lead II; respiration. a) Before feeding with cholesterol and 6-methylthiouracil, at rest; b) after effort; c) 2 weeks after cessation of feeding, at rest; d) after effort.

SUMMARY

In dogs with experimental atherosclerosis the decrease of the ECG voltage was greater and occurred more often in the first lead. Changes and inversion of the T wave, displacement of the ST interval above and below the isoelectric lines, extrasystolic arrhythmia, and signs of atrioventricular block were also noted here. The BCG showed a reduction of the total amplitude, sloping of the BCG wave complexes, and early M, splitting of wave I, and deepening of the K. The changes in the ECG and BCG pursued the type observed in patients suffering from coronary insufficiency. The velocity of the pulse wave spread remained unchanged and any alterations therein apparently set in only at the later stages of atherosclerosis.

LITERATURE CITED

1. A. V. Vinogradov, Terap. Arkh., No. 1, 76 (1952).
2. L. K. Lakshina, Evaluation of Sclerosis of the Great Vessels by the Velocity of Spread of the Pulse Wave (Candidate's dissertation) [in Russian] (Moscow, 1957).
3. A. L. Limcher, Functional Investigations in the Theory and Practice of Medical Assessment of Fitness for Work [in Russian] (Moscow, 1958), p. 52.
4. A. V. Mareev, Byull. Éksp. Biol. i Med. No. 1, 102 (1958).
5. V. N. Mentova and Z. T. Samoilova, Abstracts of Proceedings of the Tenth Scientific Session of the Institute of Therapy of the AMN SSSR [in Russian] (Moscow, 1959), p. 15.
6. V. V. Parin and A. V. Mareev, Atherosclerosis and Coronary Insufficiency (Moscow, 1956) p. 235.
7. C. J. Wiggers, The Dynamics of Circulation [Russian translation] (Moscow, 1957) p. 13.
8. A. N. Fedoseev, Byull. Éksp. Biol. i Med. No. 12, 101 (1958).*
9. G. Wakerlin, and others., Circulation 5, 426 (1957).

* Original Russian pagination. See C.B. Translation.

THE MECHANISM OF THE ELECTROCARDIOGRAPHIC DISORDERS IN ANAPHYLACTIC SHOCK

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The essential participation of the sympathetic nervous system in the pathogenesis of anaphylactic shock has been proved by the work of several authors [15 and others].

In animal experiments, Yu. S. Tatarinov [15] found that the preliminary injection of adrenalin makes the course of the shock milder. After perfusion of the isolated heart of sensitized rabbit with adrenalin, N. P. Mordovskii [8] observed modification of the reaction to the subsequent injection of serum. S. M. Pavlenko [9] and I. Kh. Abdullaev [1] showed that atropine had a moderating action in shock in guinea pigs and rabbits. A. D. Ado[2] found that the effect of bilateral vagotomy depends on the period of sensitization. A. M. Bezredka [3], M. L. Garmasheva [4], A. N. Gordienko [6], V. S. Mats-Rossinskaya [7] and others have noted the action of anesthesia in preventing anaphylactic shock.

In the present investigation we studied the part played by the sympathetic nervous system in the changes in the activity of the heart during anaphylactic shock and the influence of ether anesthesia on these changes.

EXPERIMENTAL METHOD

The part played by the sympathetic nervous system in the disorders of cardiac activity in shock was investigated in 27 guinea pigs (males) weighing 300-350 g. The animals were sensitized by a single subcutaneous injection of 0.05 ml of normal horse serum, without preservative, 15-17 days before the assaulting injection of 1 ml of the same serum into the external jugular vein. In the first series of experiments the animals received injections of ephedrine in doses of 1-4 mg/kg into the jugular vein 5-10 min before the assaulting dose; in the second series nine guinea pigs received intravenous injections of atropine in doses of 0.1-5 mg/kg 5-25 min before the assaulting dose; in 12 animals in the third series of experiments, bilateral vagotomy was carried out 5-25 min before the assaulting injection. The action of anesthesia was studied in 28 guinea pigs sensitized in the manner described above. An assaulting dose (0.2-3.5 ml of the same serum) was injected into the jugular vein of the animals on the 15th-17th day of sensitization 5-10 min after the induction of deep ether anesthesia. In order to provide a comparison with the usual course of shock, the results of 56 experiments carried out earlier were used.

In the course of each experiment simultaneous recordings were made of the electrocardiogram in three standard leads and of the respiration by means of a 4-channel ink-recording apparatus of type 4-PFD-7. In some animals an effective sign of respiration—the movement of air in the trachea—was recorded by a photocell. Simultaneously, the respiratory movements, the blood pressure in the carotid artery (by means of a Hürthle manometer) and the general motor reaction were recorded on a kymograph.

EXPERIMENTAL RESULTS

In the experiments using ephedrine and atropine, and also after vagotomy, the injection of an assaulting dose of serum into the sensitized animals caused fatal anaphylactic shock. In the period of injection of the antigen, the changes in the blood pressure and the ECG (Fig. 1b) were the same as in ordinary sensitized guinea pigs. Those observed included a slowing of the rhythm, lengthening of the QT (more rarely, the PQ) interval, left axis deviation of the T wave and an increase in its amplitude with a less marked right axis deviation of the QRS (R), demonstrating that the disorder of function mainly affected the right ventricle [5]. Similar changes in the ECG were observed during stimulation of the vagus nerves with an alternating current. In all the experiments, during the development of the shock, the T wave was displaced to the right of the axis and inverted in the standard leads; right axis deviation of the T wave from 90° ($-T_1$) was noted in three cases, from + 150° ($-T_{1,2}$) in six and beyond −150° ($-T_{1,2,3}$) in 17 cases. At the same time there was an increase in the amplitude of the T wave and a sloping negative displacement of the ST interval. These changes, reflecting a disturbance of the functions mainly of the left ventricle, were intensified in the period of respiratory-motor disturbances of shock. During the

development of shock in the sensitized animals, inversion of the T wave was observed, as a rule, in only one of the standard leads, but the magnitude of the T wave and of the discordant displacement of the ST interval was less strongly and less constantly altered.

Ventricular extrasystoles, which are customary in shock, were much reduced or absent in these particular experiments. Another characteristic feature was the acute development of terminal changes in the ECG, occurring simultaneously with the terminal disorders and cessation of the respiratory movements, but with no definite connection with the reduction of the effective sign of respiration (Fig. 1c). These changes took the form of an increasing widening of the PQ interval, an accelerated left axis deviation of the T wave and the vector of the deflection of the ST interval (with suc-

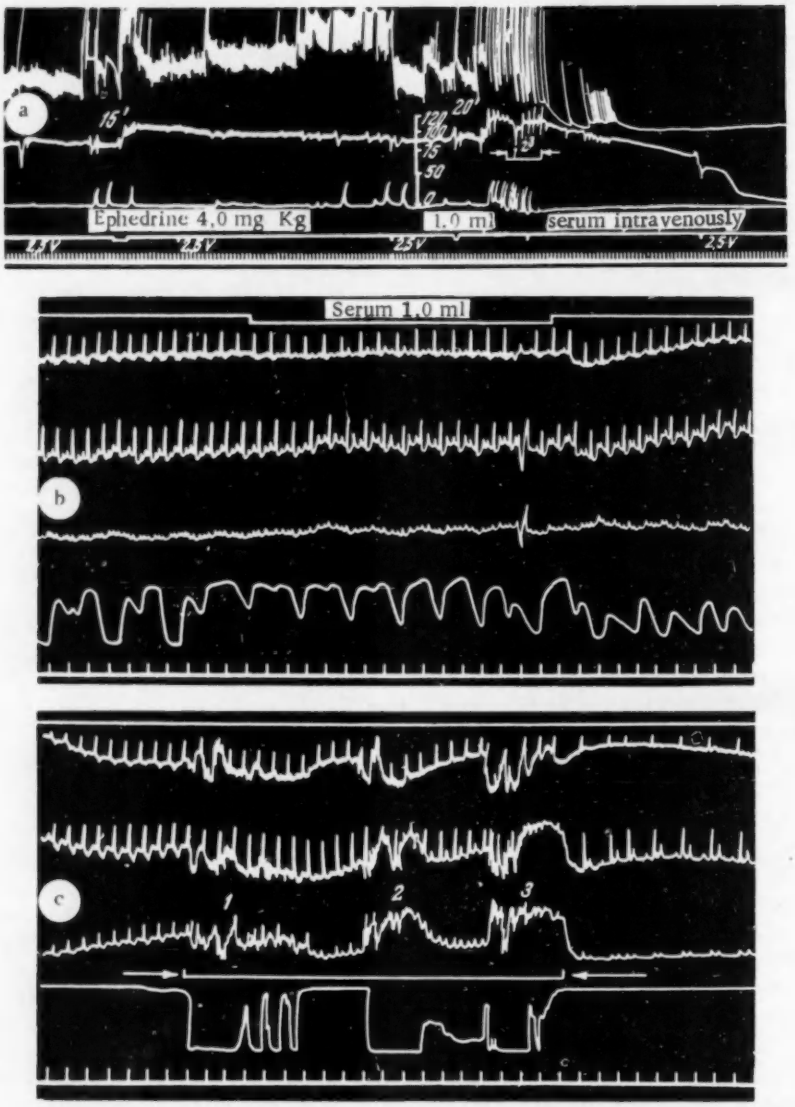

Fig. 1. Anaphylactic shock on a background of the action of ephedrine. Significance of the curves (from above, down): for a: Respiratory movements; arterial pressure; general motor reaction; marker of injection of drugs and stimulation of the peripheral end of the right vagus nerve; time marker (3 sec); for b and c: marker of injection of serum; I, II, III standard ECG leads; respiration (tracing from a piezoelectric pickup); time marker (0.25 sec). The signs →⟵← in Figs. a and c designate the same moments of the state of shock.

cessive positive transformation of T_3, $T_{2,3}$ and then $T_{1,2,3}$), and a decrease in the amplitude with analogous positive transformation of the ST deflection. Under these circumstances the ST deflection changed from concordant-negative to concordant-positive, which corresponded to the completion of the diametrical anticlockwise shift of the ST vector in the shock period. The positive transformation of the T wave was completed after the accelerated disorder of atrioventricular conduction, arising at first in the form of an incomplete, and then a complete, block. At the same time there was an increase in the amplitude of the positive T wave and of the deflection of the ST interval, and a widening of the QT interval was observed. The approximation of the electrical axes of R and T, observed in these conditions, was evidence of the disappearance of signs of a lesion predominantly of the left ventricle.

In ordinary shock the corresponding terminal changes in the ST—T complex develop less acutely This is due to the smaller divergence of the axes of the initial and terminal parts of the ventricular complex during the development of these disturbances. Disturbance of atrioventricular conduction under these circumstances takes place through a stage of dropping out of ventricular complexes, or Wenckebach-Samoilov periods.

The heart lesions in the experiments on sensitized guinea pigs using ephedrine, atropine and vagotomy were more severe than those in ordinary shock, as was shown in particular by the more profound changes in the ECG in the period before death. Furthermore, morphological investigations of the heart of a number of animals from all three series confirmed the great severity of the vascular and dystrophic lesions of the myocardium. The period between the arrest of respiration and the cessation of the working of the heart in five experiments using atropine, and in all 12 experiments with preliminary vagotomy was, on the average, shortened by half (its duration was approximately equal to 10 min). The ECG in the period before death in such cases was atypical in character; this took the form of a more pronounced slowing of the rhythm of the ventricles, a widening of the QT interval and a considerable divergence of the axes of the initial and terminal parts of the ventricular complex. The latter was connected with changes in the source of automatism and with disturbances of intraventricular conduction. The deflection of the ST interval was insignificant, and no merging of the elements of the ventricular complex into a monophasic curve took place. The ventricular complexes came to an end even when their amplitude was relatively high. The atrial rhythm was slower and irregular, and their activity ceased before or soon after disappearance of the ventricular complexes.

Of the 28 animals in which anaphylactic shock was produced on a background of deep ether anesthesia, 14 died, and no obvious relationship was observed between the outcome of the shock and the size of the assaulting dose of antigen.

The injection of serum against a background of anesthesia was accompanied by the same changes in the ECG as during the ordinary development of shock. Both external and electrocardiographic manifestations of anaphylactic shock were weakened, which is in agreement with the findings of Mikulicich [22] obtained in rabbits under nembutal anesthesia; in this case the ventricular extrasystoles were less marked or were absent altogether.

A moderate change in the size of the waves and a discordant deflection of the ST interval were observed, which developed more slowly and increased immediately before the cessation of respiration.

Terminal disorders of the ECG, similar to the same disorders in ordinary shock, developed at the same time as the cessation of the functions of the respiratory center. In this case we could find no relationship between the disorders of the ECG and the depth of the changes in blood pressure and effective respiration (Fig. 2a and b). The development of atrioventricular block was preceded by lengthening of the PQ interval; in some cases Wenckebach-Samoilov periods developed. After the arrest of respiration and the approximation of the blood pressure to the zero line, the usual signs of clinical death took place: disturbance of atrioventricular conduction, approximation of the axes of the R and T waves, increase in the amplitude of the positive ST—T complex and a reduction in the R wave, which corresponded to the formation of a monophasic curve.

In five experiments which terminated fatally, and four cases of nonfatal shock, reversal of the development of the terminal changes in the ECG took place. A minute or more after the arrest of respiration it recommenced, with a lower effective value of the respiratory movements. In the first few seconds after the first inspiration, regression of the terminal changes in the ECG and an increase in the blood pressure to (or slightly above) the initial level were observed (Fig. 2c). On the ECG, atrioventricular conduction was restored through a stage of lengthening of the PQ interval. At the same time there occurred a rapid reduction in the amplitude of the T wave and the deflection of the ST interval, and a shortening of the QT interval. Usually after 10-20 cycles, the principal disorders of the ECG were rectified. The rise in the arterial pressure was unstable; it quickly changed into a gradual fall to the zero line, but no obvious disturbances were observed in the ECG at this time. In fatal cases of shock repeated terminal disturbances of the ECG developed at the same time as the cessation of respiration. The sequence of changes in the ECG during the development and regression of the terminal disturbances was constant. Changes in the ST—T complex appeared earlier and disappeared later. Atrioventricular block and widening of the QT interval began later and disappeared sooner.

The rapid restoration of the functions of the heart simultaneously with the first, barely effective respiratory movements after prolonged and complete asphyxia and gross hemodynamic disorders can hardly be due to abolition of the myocardial anoxia. In the same way, the terminal disturbances in the ECG cannot be explained by the development of anoxia.

It must be mentioned that the idea of a direct reflex connection between the respiratory and cardiac centers had been put forward by Binet and Strumza [17]. M. I. Rafiki [13] also explains the coincidence of the terminal changes in the ECG and the cessation of respiration in shock in rabbits by disturbances of extracardial regulation.

As we have already stated above, in our experiments with vagotomy and atropinization, special changes were observed in the ECG of sensitized guinea pigs throughout the whole period of shock and death. This is evidence

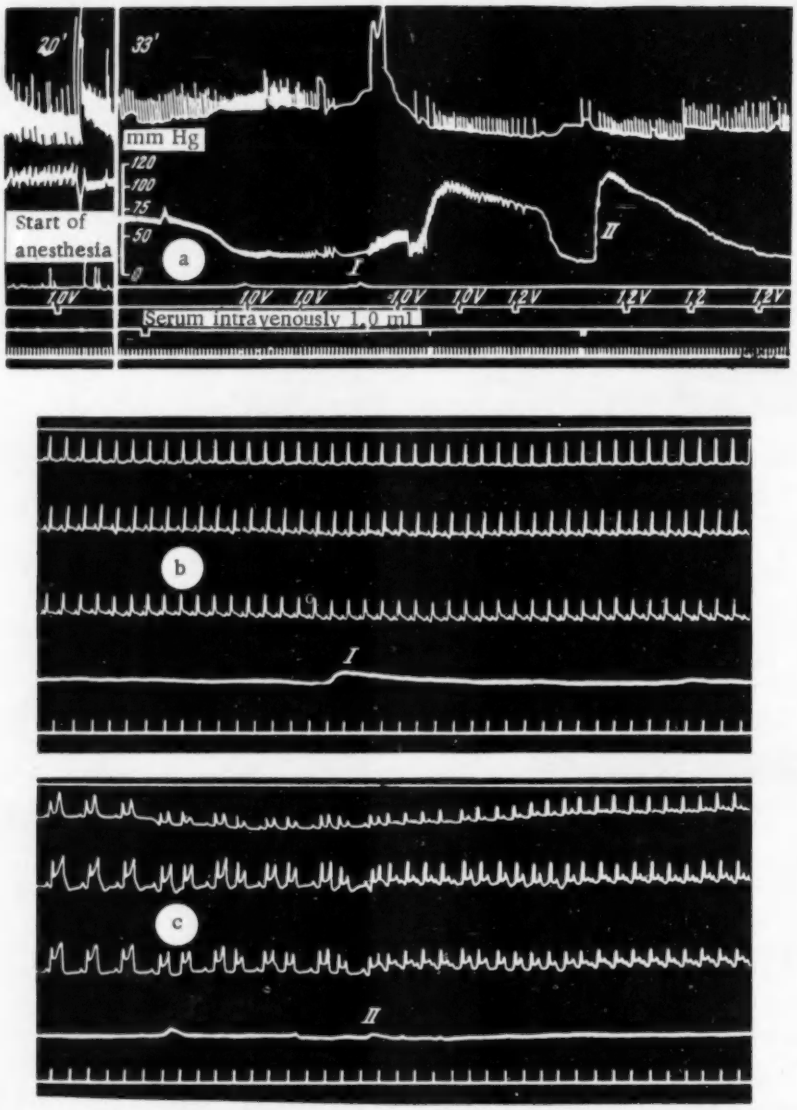

Fig. 2. Anaphylactic shock on a background of ether anesthesia. Significance of the curves (from above, down): for a: Respiratory movements; arterial pressure; general motor reaction; marker of stimulationof the right peripheral segment of the vagus nerve; marker of injection of assaulting dose of serum; time marker (3 sec); for b and c) marker of injection of serum; standard leads I, II, III of the ECG; effective respiration; time marker (0.25 sec). Points I and II on Figs. a, b, and c correspond to the same moment of the state of shock.

of the essential participation of the vagus nerves in the disorders of cardiac activity in anaphylactic shock, which is also in agreement with the findings of M. I. Rafiki [13], Criep [18] and others. The principal distinctions of the ECG in shock in these conditions (negative ST–T complex in standard leads) are a characteristic expression of sympathetic influences on the heart [12, 16, 19, 20, 21]. These disturbances may thus be attributed to the predominance in shock of sympathetic influences, intensifying the anoxia of the myocardium [11, 21].

Meanwhile, the experiments described justify the rejection of a direct role of the vagus nerves in the reflex mechanism of production of the terminal changes in the ECG, since both after atropinization and after vagotomy these disturbances develop in an even more acute and profound form. Our experimental results show that even with the most trivial changes in the rhythm of the heart and the level of the blood pressure in the course of terminal changes in the ECG, the first to disappear are the inversion of the T wave and the negative deflection of the ST interval, which are characteristic of the influence of the sympathetic nervous system.

In the course of regression of the terminal disorders, changes arise which are typical of the action of factors stimulating the sympathetic nerves: atrioventricular conduction is restored [11], systole is shortened [10, 16] and the T wave is inverted [21 and others], in conjunction with activation of respiration (Kamis-Krestovnikov phenomenon) and a rise in blood pressure. The development and regression of the terminal changes in the ECG may thus be regarded, in the first case as the expression of a fall, and in the second case of restoration, of the adaptational and trophic sympathetic influences on the heart, mainly reflex in character (ephedrine aggravates only the terminal changes in the ECG).

The greater severity of the functional and morphological lesions of the heart in animals with shock after vagotomy or administration of atropine and ephedrine is obviously the results of the predominance of sympathetic influences on the heart; this is in agreement with the findings of I. P. Pavlov [10] and Gaskell concerning the negative after-action of the accelerator nerves on the heart when nutrition is impaired.

The acute development of the terminal disturbances of the ECG in the experiments described may be explained by the considerable severity of the heart lesions arising in connection with the intensification of its work in the pathogenic conditions of anaphylactic shock. These lesions are responsible for disturbances of cardiac activity on account of a fall in the adaptational and trophic sympathetic influences on the heart.

SUMMARY

Participation of the sympathetic nervous system in the cardiac changes occurring in anaphylactic shock was studied on 27 guinea pigs, and the effect of ether anesthesia on 28 animals. Simultaneous recordings of the 3 standard ECG leads were taken as well as of respiration, blood pressure and motor reaction. The prevalence of sympathetic effects during shock was created by the administration of ephedrine, or atropine, or vagotomy. Nonspecific ECG changes, similar to the vagus-stimulation effect occurring during the intravenous booster injection of the antigen, developed both in the vagotomized and in the atropinized animals. Ventricular extrasystoles diminished in both groups of experiments. The ST vector experienced consecutive shifts to the left during the shock. The terminal disturbances of the ECG in the form of increasing positive values with rising ST complex and T wave, widening of the QT interval and disturbances of atrioventricular transmission developed simultaneously with the arrest of respiration in both vagotomized and atropinized animals. This excludes the leading role of the vagus nerve in the appearance of such disturbances. In 9 cases of shock against the background of anesthesia, a spontaneous regression of the terminal disturbances of the ECG occurred simultaneously with the resotration of respiration. The terminal ECG disturbances are evidently reflex in origin and are connected with diminished adaptational and trophic effects of the sympathetic nervous system on the heart. The regression of the terminal changes is in keeping with the degree of restoration of these influences.

LITERATURE CITED

1. I. Kh. Abdullaev, Izvest. Akad. Nauk. Uzb. SSSR, Ser. Med. No. 3, 69 (1958).
2. A. D. Ado, Antigens as Extraordinary Stimuli of the Nervous System [in Russian] (Moscow, 1952).
3. A. M. Bezredka, Anaphylaxis and Antianaphylaxis [in Russian] (Moscow, 1928).
4. N. L. Garmasheva, Mechanism of Pathological Rections [in Russian] (Leningrad, 1947) Nos. 9-10, p. 29.
5. A. V. Gol'tsman, A New Method of Analysis of the Electrocardiogram—Axonometry—and its Importance in the Clinical Management of Diseases of the Myocardium (Candidate's dissertation) [in Russian] (Odessa, 1950).
6. A. N. Gordienko, The Nervous System and Immunity [in Russian] (Krasnodar, 1949).
7. V. S. Mats—Rossinskaya, Role of the Central Nervous System in the Mechanism of Anaphylaxis (Candidate's dissertation) [in Russian] (Stalino, 1955).
8. N. P. Mordovskii, Med. Byull. Irkutsk. Med. Inst. No. 5, 18 (1942).
9. S. M. Pavlenko, Problems of Rheumatism [in Russian] (Moscow-Leningrad, 1936) Nos. 9-10, pp. 23-31.
10. I. P. Pavlov, Complete Collected Works, 1 [in Russian] (Moscow-Leningrad, 1951), p. 419.

11. V. Raab, Progress in Cardiology [in Russian] (Moscow, 1959), p. 67.
12. M. E. Raiskina, Klin. Med. No. 9, 57 (1955).
13. M. I. Rafiki, Uchenye Zapiski LGU, Ser. Biol. No. 176, 203 (1954).
14. N. N. Sirotinin, Fundamentals and Achievements of Modern Medicine 2, [in Russian] (Kharkov, 1934) p. 28.
15. Yu. S. Tatarinov, Trudy Astrakhansk. Med. Inst. 11, 191 (1954).
16. L. I. Fogel'son, Clinical Electrocardiography [in Russian] (Moscow, 1957).
17. L. Binet and M. V. Strumza, Compt. rend. Soc. biol. 141, 251 (1947).
18. Z. H. Criep, Arch. Intern. Med. 48, 1098 (1931).
19. E. Goldberger, Unipolar Lead Electrocardiography and Vectorcardiography (Philadelphia, 1954).
20. A. Grishman, and L. Scherlis, Spatial Vectorcardiography (Philadelphia, 1952).
21. E. Lepeschkin, Modern Electrocardiography (Baltimore, 1951).
22. G. Mikulicich, J. Allergy 22, 249 (1951).

BLOOD CHANGES DURING ELECTRICALLY INDUCED SLEEP

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Recent years have seen the introduction of electrical sleep therapy into clinical practice in the Soviet Union, induced by the action of a weak, pulsed, low-frequency current on the brain. Depending on the intensity and the extent of the sleep inhibition, in the overwhelming majority of patients a state of drowsiness or of sleep only slightly different from natural sleep is produced under these conditions.

It is of great interest to study the vegetative changes taking place in the body during electrically induced sleep. The character of the hematological changes arising in these conditions has been almost completely neglected in the Soviet literature, and even more so in the foreign literature.

EXPERIMENTAL METHOD

Studies of the blood picture during a course of electrically induced sleep were made in the psychoneurological division of the hospital. In all, 30 observations were made on 19 patients aged from 30 to 54 years. The diagnosis of a neurasthenic syndrome was established in all the patients; in addition two suffered from hypertension and one from cerebral arteriosclerosis.

Electrical sleep was induced in a specially equipped ward, by means of the apparatus devised by the Research Institute of Experimental Surgical Apparatus and Instruments. A constant current of low power (of the order of 0.1-1.5 ma, at a frequency of 60-70 cps) caused no unpleasant sensations in the patients, who were subjected to it for about 1½ hr. The majority fell asleep, on the average, after 20 min. Sleep lasted for about an hour.

Blood for examination was taken three times from each patient: before the current was switched on, 20-30 min after the patient had been put to bed, and then approximately one hour later, when the state of drowsiness had passed into a deeper sleep, and lastly when the current had been switched off and the patient was awake. Taking the blood lasted 4-5 min and was done with as little disturbance of the patient as possible. When taking the first sample of blood we made a deeper puncture of the pulp of the finger than usual. For this purpose we used a Frank's needle with a finely pointed stylet. Immediately after taking the blood, we applied cotton wool soaked in heparin solution to the site of puncture and fixed it to the finger with a strip of adhesive plaster. By this means we avoided the necessity for further puncture of the finger in taking the subsequent samples of blood. The depth of sleep varied from a slightly drowsy state to a profound sleep with a corresponding general relaxation of the skeletal musculature, snoring and so on. When blood was taken in deep sleep, in some cases the sleep was disturbed to some degree. The reaction of the sleeping subject to the taking of blood, in conjunction with other signs of the depth of sleep and the results of interrogation of the subjects at the end of the session, enabled the depth of sleep to be estimated.

EXPERIMENTAL RESULTS

The results of our observations on the general condition of the patients during electrically induced sleep corresponded to those reported in the literature. The respiration as a rule became slower and deeper, the pulse was slowed by several beats per minute and the arterial pressure fell on the average from 128/81 to 117/75 mm Hg. The body temperature fell on the average by 0.2°. After the procedure the patients volunteered that they felt better, more wide-awake and tranquil.

The dynamic investigation of the blood revealed a definite relationship between the changes in certain indices. It was found in the first place that the character and degree of the hematological changes were closely connected with the function of sleep itself and with its qualitative features. This can be seen from a comparison of the results of investigation of the blood during tranquil, deep sleep (16 observations) with those of cases in which the patients were not asleep at the time of the procedure, but were in a drowsy state (14 observations). Assuming that the mean error of the method when counting the leukocytes to be ± 7 %, and the red cells ±2.5 %, we found that during deeper sleep the leukocyte count progressively fell, on the average, by 25 %. The fall in the leukocyte count was mainly at the expense of the neutrophils and lymphocytes (see Table and Figure). As a result of the sleep the red cell count and the hematocrit index rose, thus showing some degree of hemoconcentration.

In the patients who were in a state of light sleep at the time of the procedure, the total leukocyte count not only did not fall but, in isolated cases, even increased.

Change in Absolute Values of Hematological Indices During Electrically Induced Sleep (in %) (Mean results of all observations)

Level of sleep	Time of examination of blood	Red cells	Hematocrit	Leukocytes	Leukocyte formula			
					eosinophils	neutrophils	lymphocytes	monocytes
Deep	During sleep	+10	+13	−25	−33	−25	−25	−25
	When awake	−6	−2	+23	−2	+20	+56	+51
Superficial	During sleep	+5	+5	+3	−30	+1	+10	+2
	When awake	−6	+4	+9	+63	+10	+5	−7

The red cell count and hematocrit index rose, but to a lesser degree than during deep sleep.

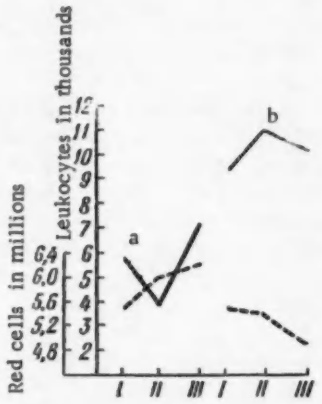
When the subjects were awake, which usually coincided with switching the current off, in spite of the fact that not more than 5-10 minutes elapsed between the discontinuation of the electrically induced sleep and the taking of the blood, the blood picture was greatly altered. In cases of deep sleep, on waking, the leukocyte count rose sharply, sometimes exceeding the initial level. The rise in the leukocyte count took place mainly on account of neutrophils and lymphocytes. In contrast to the leukocytes, the red cell count fell on the average. During the awakening of those subjects who, in the course of the session were in a state of light sleep or drowsiness, no rise in the total leukocyte count was observed. The red cell count showed a general tendency to fall.

Analogous changes in the blood picture also take place during natural sleep. One of the authors of the present article [1, 2] observed that, as a result of tranquil, deep sleep, the leukocyte count in healthy persons regularly falls, on the average, by 30%. Restless, superficial natural sleep is characterized by the absence of fall of the leukocyte count and, in individual cases, even by a rise in the number of leukocytes. In this case the red cell count rises, but to a lesser degree than during deep sleep. The hematocrit index is also increased as a result of natural sleep.

All the changes mentioned above are, of course, within physiologically normal limits of variation. In deep sleep they reflect the general tendency of the hematological indices to even themselves out and to approximate themselves to normal, which ultimately bestows on sleep the character of a restorative process.

The identical nature of the hematological changes is evidence that what has been said applies equally to electrically induced sleep, which has no negative influence whatever on the blood system, but, on the contrary, causes changes analogous to those which develop during natural sleep. This affords further proof of the close relationship between the two types of sleep and, consequently, justifies the wider use of electrically-induced sleep for clinical purposes.

The fact that the blood picture changes during electrically induced sleep, by comparison with the initial data, the converse nature of the changes on waking, and the relationship between the hematological changes and the quality of the sleep all suggest that the changes observed are connected with the function of sleep itself. We are thus correct in assuming that the changes in the blood picture during electrical sleep are due to changes in the functional state of the central nervous system. The direction and degree of the hematological changes are evidently dependent on the intensity and the extent



Changes in the total leukocyte and red cell counts during electrically induced sleep (observation on May 14, 1958). a) Deep sleep; b) state of drowsiness. Time of investigation of blood: I) Before session of electrical sleep; II) during session; III) at conclusion of session. —) leukocytes; -----) red cells.

of development of the process of inhibition and of those functional dynamic relationships which are formed under these circumstances between the cerebral cortex and the subcortical structures directly concerned with the change in the morphological composition of the peripheral blood.

SUMMARY

The change in the blood picture during electrically-induced sleep is associated with the function of the sleep as such. In deep sleep the number of leukocytes falls progressively at the expense of all the white blood cells. There is a rise in the number of erythrocytes and the hematocrit index. In a state of drowsiness the number of leukocytes does not drop and in individual instances it even rises; the erythrocyte count and hematocrit index are not increased so much as in deep sleep. On awaking, the leukocytic shift assumes a character which is just the opposite of that noted during profound sleep. In general, the fluctuations in hematological indices are within physiologically normal limits and analogous to those observed in natural sleep.

LITERATURE CITED

1. M. I. Ul'yanov, Transactions of the Third All-Union Conference of Laboratory Physicians [in Russian] (Moscow, 1955) p. 251.
2. M. I. Ul'yanov, Byull. Éksp. Biol. Med. No. 1 (Supplement), 45 (1957).*

* Original Russian pagination. See C.B. Translation.

FUNCTIONAL STATE OF THE ADRENAL CORTEX IN EXPERIMENTAL HEMOLYTIC ANEMIA DUE TO PHENYLHYDRAZINE

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Steroid hormones from the adrenal cortex have been widely used in recent years in the treatment of the acquired hemolytic anemias [1, 2, 3, 5]. It has been suggested that the therapeutic action of the adrenal steroid hormones in this group of diseases is the result of depression of the functions of the reticuloendothelial system, where anti-red cell antibodies are produced. We know from the literature, however, that the steroid therapy of the hemolytic anemias is not always accompanied by obvious improvement. When administration of the adrenal hormones is discontinued, recurrence of the disease is common [3]. Satisfactory results from the treatment of the acquired hemolytic anemias with steroid hormones, just as in the course of the disease before treatment is discontinued, are to a large extent dependent on the reactivity of the patient and, in particular, on the functional state of the adrenal glands.

In the literature known to us, we were unable to find data concerning the function of the adrenal cortex at various periods in the course of the acquired hemolytic anemias. We therefore decided to make an experimental study of the functional state of the adrenal cortex in animals with hemolytic anemia due to phenylhydrazine. There are reports in the literature that the development of anemia in animals poisoned with phenylhydrazine, as in cases of acquired hemolytic anemia, is based upon autoimmune mechanisms [6, 7].

EXPERIMENTAL METHOD

Experiments were carried out on 84 white rats of both sexes, weighing 110-280 g. The animals were kept on a balanced diet. Anemia was induced by a single subcutaneous injection of a 2% solution of phenylhydrazine hydrochloride in a dose of 40 mg/kg. The blood picture and the functional state of the adrenal cortex were investigated after 3, 6, 12, and 24 hr and on the 2nd, 4th, 6th, 9th, and 13th days after injection of phenylhydrazine. Estimations were made of the red cell count, the blood hemoglobin concentration, and the red blood picture in films. The hematological investigations were made by the usual methods. In order to define the functional state of the adrenal cortex the following tests were used: the eosinophil reaction of the blood, the change in weight of the adrenal glands and the estimation of the free ascorbic acid in the adrenals. Histological studies of the adrenals were also carried out. The eosinophils were counted by Dunger's method. The content of free ascorbic acid in the adrenal tissues was estimated by titration of an extract of the adrenals with 2,6-dichlorophenolindophenol. For the histological study of the adrenals, films stained with hematoxylin and eosin were used.

EXPERIMENTAL RESULTS

As the figures in the table show, the phenylhydrazine anemia reached its maximum on the fourth day. At this time the red cell count fell to 59.5 %, and the hemoglobin concentration to 63.2 % of the mean blood level in the intact animals. The red blood picture in the film showed severe anisocytosis, macromicrocytosis, severe polychromatophilia and the presence of solitary normoblasts and red cells with Jolly bodies.

TABLE. Composition of the Peripheral Blood and Indices of the Functional State of the Adrenal Cortex at Various Stages of Hemolytic Anemia Due to Phenylhydrazine.

No. of expt. animals	Time elapsing after injection of phenylhydrazine	Mean values of indices studied (as % of controls)				
		red cell count	hemoglobin concen.	eosinophil count	weight of adrenals	ascorbic acid content
5	3 hours	–	–	79.5	–	–
5	6 "	–	–	23.1	–	–
7	12 "	–	–	208.5	–	–
7	24 "	–	–	121	–	–
10	2 days	77.2	60.0	192	100	114
10	4 "	59.5	63.2	198	100	106
10	6 "	68	63	139	120	86.5
10	9 "	77.9	89.5	75.4	135	85.4
10	13 "	84.5	93.8	62.5	110	99

Starting on the sixth day of the observations, signs of regeneration of the red blood were observed. On the 13th day after injection of phenylhydrazine, the red cell count reached 84.5 % and the hemoglobin concentration in the blood 93.8 % of that in the controls. By the ninth day of the experiment, no abnormalities could be detected in the red blood picture in the films of 5 of the 10 experimental animals. On the 13th day no abnormalities in the morphological structure of the cells were observed in the blood films of all the experimental animals.

In the course of the first six hours after the injection of phenylhydrazine, when the signs of hemolysis of the red cells were still slight [4], the character of the eosinophil reaction of the blood gave evidence of the participation of the adrenal cortex in the pathogenesis of the developing anemia. Two phases were discerned in the reaction of the eosinophils to the injection of phenylhydrazine. In the course of the first six hours, to judge by the developing eosinopenia, the functional activity of the adrenal cortex was increased. It may be considered that this was the normal reaction of the adrenals to an inadequate stimulus, in this case the procedure of injection of phenylhydrazine. Twelve hours after injection, the increase in the functional activity of the adrenal cortex was replaced by a decrease. The number of eosinophils in the peripheral blood rose to 208.5 %. At the end of the first 24 hours of the experiment a further increase in the activity of these glands was observed, but to a lesser degree than during the first six hours. The number of eosinophils in the peripheral blood at this time fell nearly to the level observed in the blood of the intact animals (121 %).

As the table shows, the development of hemolytic anemia was accompanied by depression of the activity of the adrenal cortex. At the height of the anemia a marked eosinophilia was observed in the peripheral blood: the eosinophil count in the blood was 192-198 % by comparison with that in the intact animals. The free ascorbic acid content of the adrenal tissue was increased by 6-14 %. No changes in the weight of the adrenals could be detected at this period.

With the commencing regeneration of the red blood, an increase in the activity of the adrenal cortex was observed, and this continued while normalization of the composition of the red blood took place. On the sixth day of the experiment the eosinophil count in the blood fell from 198 to 139 % and continued to fall further, so that on the ninth day it was 75.4 %, and on the 13th day, 62.5 % of the level in the blood of the intact animals. The increase in the functional activity of the adrenal cortex was accompanied by an increase in the weight of the glands. Six days after injection of phenylhydrazine, the weight of the adrenals in the experimental animals was, on the average, 20 % higher than that in the intact animals, and after nine days it was 35 % higher. The ascorbic acid content of the adrenal glands on the sixth to the ninth day after injection of phenylhydrazine was increased by 14-15 % by comparison with its content in the adrenal glands of intact animals. On the 13th day of the experiment the ascorbic acid content in the adrenals of the experimental animals was the same as in the intact animals. However, the observed increase in weight of the adrenals and the eosinopenia of peripheral blood were evidence of the increased activity of the adrenal cortex.

Microscopic examination of adrenal preparations taken on the second, fourth and sixth days of the experiment showed well-marked signs of cloudy swelling of the cells of the zona fasciculata of the adrenal cortex. The microscopic picture of the adrenal glands on the ninth and 13th days showed no essential abnormalities by comparison with the morphological state of the adrenal glands of the intact animals.

SUMMARY

A study was conducted of the functional condition of the adrenal cortex in rats at various stages of experimental hemolytic anemia. With the development of anemia the functional activity of the adrenal cortex was depressed. Restoration of the morphological composition of the blood was accompanied by a rise of the functional activity of the adrenal cortex.

LITERATURE CITED

1. A. Anastasov, Problemy Gematol. No. 4, 3 (1958).
2. I. A. Kassirskii and G. A. Alekseev, Clinical Hematology [in Russian] (Moscow, 1955).
3. Yu. I. Lorie and M. D. Patsiora, Problemy Gematol., No. 1, 3 (1958).
4. Ya. Ya. Sokolovskaya, Arkh. Patol. No. 4, 9 (1948).
5. W. Dameshek, Blood 5, 779 (1950).
6. H. Luginbühl, Schweiz. med. Wschr. 87, 1251 (1952).
7. F. Muratore and N. Vulpis, Haematologica 37, 1275 (1935).

THE REACTION TO BLOOD LOSS OF VARYING DEGREE DURING EXPERIMENTAL NONINFECTIOUS PERITONITIS

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Inflammation of the peritoneum occupies an important place in clinical practice; it is especially common to observe a combination of peritonitis with blood loss. The complex of defensive and adaptive reactions, developing in response both to peritonitis and to blood loss, occasionally run their course against a background of modification of reactivity caused by each of these processes.

This provides an explanation of the influence of blood loss on the course of inflammatory processes, which is known from clinical and experimental observations [1-5].

In the present research we attempted to examine the development of certain compensatory defensive reactions in peritonitis complicated by blood loss.

EXPERIMENTAL METHOD

Experiments were carried out on 50 rabbits, which were divided into five groups, with ten animals in each group.

The first group consisted of healthy animals, and were controls; the second group, of rabbits with peritonitis but without blood loss; the third, of rabbits with peritonitis and with loss of 5% of blood; the fourth, of rabbits with peritonitis and a 10 % blood loss, and the fifth, of rabbits with peritonitis and a 15 % blood loss. Before the experiment the animals were kept for two days under identical conditions and on the same diet. In order to produce peritonitis, under aseptic conditions the rabbits were given an intraperitoneal injection of a 20 % peptone solution, in a dose of 4 ml/kg body weight. In the experiments with bleeding, the blood was taken from the chamber of the heart, and the total blood mass was taken to be one thirteenth of the body weight. Indications of the development of peritonitis were the body temperature, leukocytosis in the blood, the presence of an exudate in the abdominal cavity and, finally, pathohistological examination of the peritoneum.

The consequences of blood loss were assessed by the decrease in the hemoglobin concentration of the blood and in the red cell count.

The animals thus prepared were examined repeatedly for one or two days; they were then subjected to the acute experiment, in the course of which we studied the reaction of the animals to the intravenous injection of the following substances: 1) a 20 % peptone solution; 2) transudate taken from patients with cirrhosis of the liver; 3) exudate taken from patients with exudative pleurisy, and 4) the rabbits' own exudate. All these substances were injected in a dose of 0.5 ml/kg body weight. Changes in the blood pressure were recorded on paper on a kymograph.

EXPERIMENTAL RESULTS

The experimental results are shown in the table.

It may be seen from the results in the table that, in the animals in which peritonitis was accompanied by blood loss, the febrile reaction was weakened in proportion to the degree of blood loss. The leukocyte reaction was stimulated by moderate blood loss, but loss of a larger volume of blood (15 %) did not have this

Indices in All Groups of Experimental Animals (mean figures)

Group	Body temperature	Leukocyte count in the blood (thoudands/mm³)	Red cell count in the blood (thousands/mm³)	Hemoglobin (Sahli units)	Initial systolic pressure (in mm Hg)
First	38,3°	8,1	4 546	75	93
Second	39,5° (+1,2°)	15,0 (+6,9)	3 758 (—788)	63 (—12)	90 (—3)
Third	39° (+0,7°)	18,9 (+10,8)	3 078 (—1468)	61 (—14)	77 (—16)
Fourth	38,7° (+0,4°)	16,7 (+8,6)	3 051 (—1495)	60 (—15)	71 (—22)
Fifth	38° (—0,3°)	14,3 (+6,2)	2 954 (—1 592)	58 (—17)	73 (—20)

effect. Analysis of the kymograms obtained showed that, in the group of control animals, the injection of peptone lowered the systolic pressure; the same phenomenon, but to a less pronounced degree, was observed after injection of the transudate and exudate from the patients, and injection of the rabbit exudate led to the greatest fall in pressure. In the rabbits with peritonitis but without blood loss, these reactions were less pronounced; in animals with peritonitis and blood loss a greater fall in pressure— both the initial pressure and that obtained in response to the injection of the various substances— was observed. The development of peritonitis evidently prevented the creation of compensatory pressor mechanisms, for the peritonitis itself caused no essential fall in pressure.

SUMMARY

An investigation was made into the development of some protective-compensatory reactions in peritonitis complicated by blood loss of various degrees (5, 10, and 15 % of the total mass). The febrile reaction in non-infectious peritonitis was reduced by blood loss. A slight blood loss stimulated leukocytic reaction; the latter was not observed in response to considerable bleeding. The pressor reactions in peritonitis were weakened by blood loss; this was especially noticeable when certain hypotensive substances (20 % peptone solution, transudate and exudate obtained from the patients, and exudate of rabbits) were injected intravenously.

LITERATURE CITED

1. N. V. Kalinina, Med. Zhur. Ukrain. 20, 63 (1950).
2. G. F. Koveshnikova, New Developments in Medicine [in Russian] (Moscow, 1951) No. 24, p. 60.
3. B. E. Linberg, Soviet Med. No. 1, 3 (1953).
4. F. S. Sikolenko, Khirurgiya No. 7, 62 (1956).
5. S. T. Frid, cited by I. R. Petrov, Khirurgiya, No. 1, 107 (1950).

CHANGES IN THE RATE OF EFFECTIVE PLASMA FLOW, THE RATE OF GLOMERULAR FILTRATION, AND THE FILTRATION FRACTION IN THE KIDNEYS DURING SERUM SENSITIZATION

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There are reports in the literature [4, 5, 12, 13, 14] that experimental nephritis and nephrosis produced in animals sensitized to foreign serum or to bacterial antigens are severer in degree than similar lesions of the kidneys in unsensitized animals. These findings are largely responsible for the statement [2, 8] that diffuse glomerulonephritis is a disease of allergic nature.

It may now be regarded as proved [1, 3, 7] that the injection of an assaulting dose of antigen into the renal artery of a sensitized animal causes the development of a hyperergic reaction similar to the picture of diffuse glomerulonephritis. This explains the attempts of research workers to define the morphological and functional characteristics of the kidneys during sensitization. We know that, in serum sensitization, changes may take place in the nitrogen-excreting capacity [11], as well as delay in the excretion of uric acid by the kidneys [1]; there are reports [6] of an increase in the rate of excretion in the urine of radioactive phosphorus P^{32}, injected parenterally into an animal.

In the light of the filtration—reabsorption—secretion theory of urine formation, modern methods of study of the functional powers of the kidneys enable the activity of different parts of the nephron to be investigated in detail.

The object of the present research was to study the rate of glomerular filtration, the rate of the effective plasma flow, and the magnitude of the filtration fraction in the kidneys of dogs sensitized to normal horse serum.

EXPERIMENTAL METHOD

The investigations were carried out on five dogs—females, weighing 14—19 kg—under chronic experimental conditions. One month before the beginning of the experiments, the ureters of each animal were transplanted to the skin of the abdomen by the Pavlov—Tsitovich method. The plan of the investigations was as follows: at 9-10 A.M. the dog was placed on the apparatus, in a fasting state; the urine was collected by means of a special funnel every 15 min; observations on the spontaneous diuresis were conducted for 30 min and then, by means of a gastric tube, the animal was given a high water intake of 60 ml/kg body weight. Next, into the dorsal lateral metatarsal vein, an infusion was given of physiological saline containing inulin (0.3 %) and cardiotrast (diotrast) (0.3 %). The concentration of inulin in the plasma under these conditions did not exceed 10 mg %, and the cardiotrast concentration—2 mg %. After perfusion had proceeded for 40-50 min, when the rate of diuresis had reached 5-8 ml/min, the rate of the effective plasma flow and the rate of glomerular filtration were determined in two 15 min clearance periods.

The inulin in the plasma and urine was determined by Harrison's [10] method and the cardiotrast by the method of Rolf and White [15]. Experiments were performed once in seven days on each animal.

In the course of three weeks the background for each dog was determined, on the basis of six clearance periods, after which the animal was sensitized to horse serum in a dose of 0.2 ml/kg body weight, given by subcutaneous injection on three occasions on alternate days. In each animal the investigations were carried out on the 7th, 14th, and 21st days after the first sensitizing injection. The presence of sensitization was established retrospectively as a result of the development of anaphylactic shock in response to the injection of an assaulting dose of serum in a volume of 0.6 ml/kg body weight. The pattern of the anaphylactic shock was recorded by means of tracings of the arterial pressure and respiration.

The following were used in the experiments: horse serum prepared by the Moscow Mechnikov Institute, series No. 861; cardiotrast manufactured by the Lomonosov Chemopharmaceutical Factory; inulin of British manufacture.

The filtration fraction was expressed in per cent and was calculated by the formula:

$$\frac{C_{in}}{C_{ct}} \cdot 100 = FF$$

Changes in the Effective Plasma Flow, Glomerular Filtration, and Filtration Fraction during Serum Sensitization

	Background	Days of sensitization		
	average value and limits of variation	7	14	21
	Dog No. 1			
C_{ct}, ml/min	319,98 (274,95—354,00)	270,60	361,87	288,76
C_{in}, ml/min	69,05 (61,92—79,30)	73,72	89,21	80,09
FF %	21,70 (18,80—23,10)	27,26	24,66	27,73
	Dog No. 2			
C_{ct}, ml/min	249,28 (216,29—279,19)	165,65	265,22	347,38
C_{in}, ml/min	69,23 (57,11—87,88)	55,37	72,37	116,89
FF %	27,65 (22,71—32,57)	33,42	27,28	33,76
	Dog No. 3			
C_{ct}, ml/min	291,68 (285,31—297,99)	279,86	205,61	306,86
C_{in}, ml/min	76,71 (71,99—85,36)	78,45	102,47	85,91
FF %	26,30 (24,47—29,56)	28,23	49,85	27,90
	Dog No. 4			
C_{ct}, ml/min	324,85 (296,64—356,40)	244,28	172,50	179,57
C_{in}, ml/min	72,53 (62,95—77,63)	55,87	67,72	63,24
FF %	22,36 (21,22—23,39)	22,88	39,39	35,45
	Dog No. 5			
C_{ct}, ml/min	321,25 (300,00—341,25)	280,34	220,84	205,22
C_{in}, ml/min	75,38 (64,15—84,38)	86,76	71,62	83,47
FF %	23,53 (18,79—27,07)	30,99	32,46	40,67

The mean background value was calculated from three experiments; C_{ct}) effective plasma flow; C_{in}) glomerular filtration; FF) filtration fraction.

where C_{in} is the glomerular filtration; C_{ct}, the effective plasma flow; FF, the filtration fraction.

EXPERIMENTAL RESULTS

Thirty experiments were carried out on five animals (59 clearance periods, including 118 clearances of inulin and cardiotrast) and the results obtained were approximately the same (see Table).

The presence of serum sensitization in all the dogs was confirmed at the end of the experiments by the development of the usual picture of anaphylactic shock after the assaulting injection of antigen.

The effective plasma flow during the period of sensitization was characterized by a decrease of 4-46 % of the mean value of the "background" in ten of the 15 experiments. At the same time a relationship was found between the number of cases of a fall in the cardiotrast clearance and the day of sensitization. For instance, on the seventh day the cardiotrast clearance was reduced in all five animals, on the 14th day—in three animals, and on the 21st day of sensitization—in two animals. An increase of 5-39 % in the cardiotrast clearance was observed in one animal on the 14th day, and in two on the 21st day of sensitization.

On the whole the rate of glomerular filtration varied within the "background" limits. At individual moments of sensitization, however, a marked increase in the inulin clearance was observed. On the seventh day, the glomerular filtration was increased in one animal, on the 14th day—in two animals, and on the 21st day of sensitization—in three animals. In dog No. 2, on the 21st day of sensitization, the inulin clearance was increased by 68%. In two dogs, Nos. 2 and 4, on the seventh day of sensitization the inulin clearance was increased by 20 %.

In the majority of experiments (in 11 out of 15) the filtration fraction was increased in the period of sensitization. On the seventh day of sensitization an increase observed in three animals, on the 14th day—in four animals and on the 21st day— in four animals. The greatest increase in the filtration fraction (by 67-89 %) was observed on the 14th and 21st days of sensitization.

In the light of modern views on the system or regulation of the circulation of blood in the kidneys, the change in the rate of the effective plasma flow, which we observed in the serum sensitization experiment, may be explained as the result of a change in the size of the lumen of the arterioles of the glomerular apparatus. A decrease in the rate of the effective plasma flow may be connected with a reduction in the lumen of the afferent and efferent arterioles. With a constant or increased rate of glomerular filtration, however, a slowing of the effective plasma flow gives evidence of the predominant constriction of the efferent glomerular arterioles. The increase in the filtration fraction supports this explanation.

The appreciable increase in the rate of glomerular filtration in several of the sensitization experiments, with no change in or a slowing of the effective plasma flow indicates the increased permeability of the glomeruli.

From a comparison of the data on the effective plasma flow, glomerular filtration, and filtration fraction, it may be concluded that constriction of the efferent glomerular arterioles took place in the sensitization experiments (in 11 of 15 experiments). Meanwhile, in two experiments (dogs Nos. 2 and 4 on the seventh day of sensitization), constriction of the afferent arterioles took place, for a decrease in the inulin and cardiotrast clearances was observed. In the overwhelming majority of experiments, sensitization thus had a marked vasoconstrictor effect. From these results it is possible to suggest an explanation of the increase in the pressor vascular reaction in the kidneys of sensitized dogs under our experimental conditions. In connection with V. M. Khramov's findings [9], showing an increase in the pressor vascular reactions in rabbits sensitized to horse serum, while the region of the kidneys in the animals was simultaneously cooled, such a hypothesis appears to us to be justified.

It has been shown [3] by morphological investigations that, during sensitization of rabbits, hyperemia of a large proportion of the glomeruli is observed. This can be explained in the light of our experimental results as the result of constriction of the efferent arterioles.

SUMMARY

Experiments were performed on 5 male dogs with ureteric orifices exteriorized into the abdominal skin. The author studied the effect of serum sensitization on the effective plasma flow (determined by means of cardiotrast), on the glomerular filtration (determined by inulin), and on the filtration in the kidneys. A reduction of cardiotrast clearance was noted in the majority of the experiments; inulin clearance was changed within limits approaching normal values; however, in individual cases it was markedly increased and as a rule the filtration fraction showed a rise.

LITERATURE CITED

1. A. Aliev, Klin. Med. No. 12, 64-67 (1939).
2. M. S. Vovsi, Problems of Theoretical and Practical Medicine, Collection 6 [in Russian] (Moscow, 1937) pp. 115-118.
3. M. S. Babitskaya, Klin. Med. No. 5, 45-50 (1940).
4. K. P. Lavrova, Arkh. Patol. Anat. 4, 41-48 (1938).
5. V. G. Molotkov, The Role of the Hemolytic Streptococcus and of Isonephrotoxin in the Pathogenesis of Acute Diffuse Glomerulonephritis (Doctoral dissertation) [in Russian] (Smolensk, 1941).
6. N. N. Partskhaladzem, The Study of the Content of Radioactive Phosphorus in the Blood and Urine of Guinea Pigs in a State of Sensitization and after Injection of an Assaulting Dose of Antigen (Candidate's dissertation) [in Russian] (Moscow, 1954).

7. S. D. Reizel'man, Klin. Med. No. 6, 689-698(1938).
8. E. M. Tareev, The Nephrites [in Russian] (Moscow, 1958).
9. V. M. Khramov, The Blood Pressure during Cooling of the Region of the Kidneys and Changes in the Reactivity of the Animal (Candidate's dissertation) [in Russian] (Odessa, 1954).
10. H. E. Harrison, Proc. Soc. Exper. Biol. and Med. 49, 111-114 (1942).
11. Z. Heilner, Ztschr. Biol. 58, 333-354 (1912).
12. C. K. McLean, J. D. L. Fitzgerald, O. Younghusband et al., Arch. Path. 51, 1-12 (1951).
13. M. Masugi and Y. Sato, Arch. path. Anat. 293, 615-664 (1934).
14. A. R. Rich and J. E. Gregory, Bull Johns Hopkins Hosp. 72, 65-88 (1943).
15. H. L. White and D. Rolf, Proc. Soc. Exper. Biol. and Med. 43, 1-7 (1940).

INFLUENCE OF ELECTROMAGNETIC FIELDS ON THE ELECTRICAL ACTIVITY OF RABBIT CEREBRAL CORTEX

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The problem of the effects of electromagnetic fields (EMF) on biological materials is very interesting, since, thanks to the development of technology, humans are increasingly subjected to the influence of such phenomena. In addition, the study of the effects on organisms of weak electromagnetic and magnetic fields is of general biological interest.

The literature contains reports on the sensitivity of various organic systems to electromagnetic fields, particularly on the sensitivity of the nervous system [1, 2, 4, 5, 6, 7].

While studying the effects of x-rays on the central nervous system of the rabbit, we discovered the following facts. In control studies in which the tube emitted neither heat nor x-rays, but with the high voltages maintained, we discovered that the electroencephalogram (EEG) of the rabbit was distinctly modified. Obviously, there could be only two causes underlying these changes: First, the effect of sound produced by the transformer or resulting from unstable leaks; second, the effect of electromagnetic oscillations produced when the tube and input cable were under high voltage.

Complete surgical bilateral exclusion of hearing did not abolish the observed effect. In addition, during "simulated" conditions which reproduced the setting without high voltage, the electrical activity of the brain was completely unaffected. Consequently, our first hypothesis could not explain the recorded phenomena. There remained the second possibility, i.e., the influence of electromagnetic fields. In connection with this, further experiments were directed to the study of the effect of EMF on the central nervous system.

METHODS AND RESULTS

During the experiments animals were placed in a shielded room. The high-voltage transformer was placed outside the room, but the single cable leading from the apparatus was inside the room at a distance of approximately 1 m from the animal. Apparently, the source of EMF was the high-voltage cable from the transformer. The cable had its own capacitance of 50 $\mu\mu$f. In addition, the apparatus had various "parasitic" capacitances shunting the casing of the high-voltage transformer, the high-voltage kenotron, etc. High-frequency oscillations could therefore result from these capacitances and leak along the high-voltage elements, particularly along the cable insulation. All our attempts, however, to determine the parameters of these oscillations (frequency, amplitude, etc.) were not crowned with success.

EMF was produced for various intervals from 8-15 sec. In order to record accurately the onset and termination of EMF, one channel of the oscillograph was connected across a resistance in parallel with the primary winding of the transformer. Biological potentials were recorded with an AC amplifier and another oscillograph channel.

Experiments were conducted on 98 rabbits. Using bipolar electrodes (interelectrode distance approximately 0.5 cm), potentials were led off from the parietal or visual area of the cortex through a trephined cranial opening 1 cm in diameter.

Reaction of the central nervous system to EMF was recorded in 84 out of 98 rabbits. Nearly all cases developed distinct depression of cortical potentials with amplitude reduction of 2–4 times. Intensification of electrical activity, indicated by the appearance of high-amplitude high-frequency oscillations, was recorded in only three animals.

The reaction of the cortex occurred immediately after the onset of EMF, in most cases during the first second (Fig. 1).

Depression of cortical activity usually lasted 2-8 sec, but in some animals it was more prolonged, lasting throughout the EMF interval or even beyond it (Fig. 1).

Frequency changes were observed in all animals. EEG frequency analysis (Bernstein-Livanov method [3]) showed that these changes usually involved the spectral range 1.3-8 cps (Fig. 2).

In order to examine precisely the relations between EMF parameters and reaction of the central nervous system, we set up experiments involving changes in EMF intensity. Both alternating and monotonic intensity

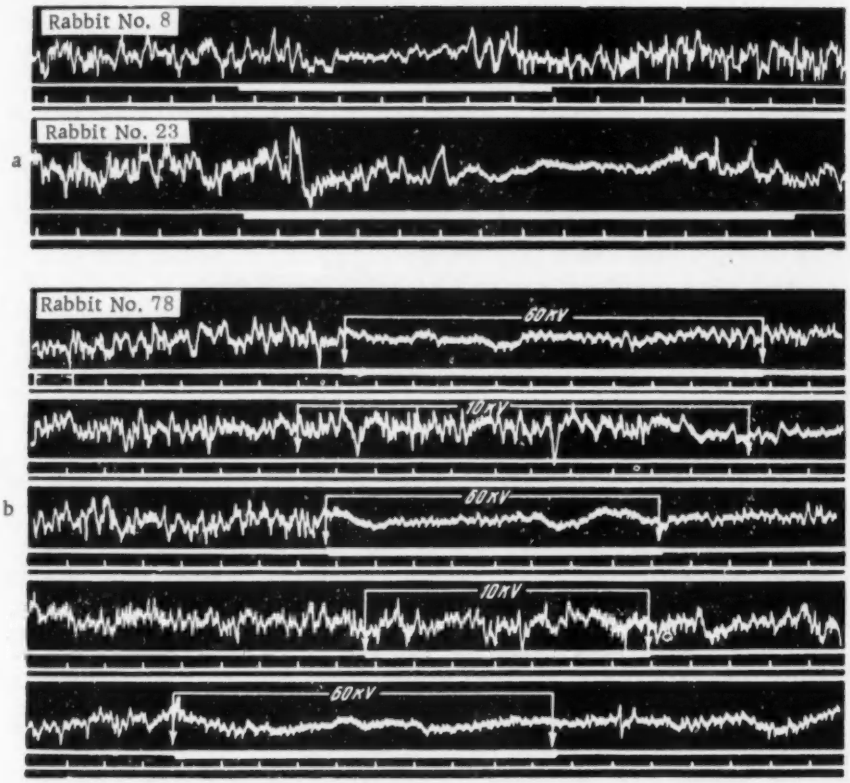

Fig. 1. a) Rabbits Nos. 8 and 23: Changes in electrical activity of the brain during the action of EMF; b) rabbit No. 78 : relation of changes in brain potentials to alternating physical parameters of EMF (10 and 60 kv). Second and third channels mark onset and termination of EMF (thickened lines) and time (1 sec).

sequences were used. Output transformer values were 10, 20, 40, 50, and 60 kv. It was found that changes became more distinct with increased voltages (Fig. 1b).

Experiments involving changes in the distance between the animal and the cable (which was the source of EMF), showed that at 2 m reaction was either absent or very weak; if the animal was placed closer to the cable, reaction intensity of EMF in turn increased the reaction of the central nervous system.

In order to determine the significance of the onset time of EMF from the moment the apparatus was switched on, we conducted experiments with intensity gradually increasing from 0-60 kv in the course of 2-3 sec. It was found that even in this case the reaction was completely maintained. Consequently, EMF is effective throughout the time the transformer is in operation.

We thus obtained experimental evidence concerning the nature of the changes in electrical activity of the cerebral cortex under the influence of EMF. The following facts support the idea that these changes are biological reactions of the brain: First, the reaction, as a rule, had a latent period of 1-2 sec, and sometimes even more; second, during prolonged action of EMF the initial abrupt change in the EEG was in most cases followed by a return to the original state; third, in narcotized animals the reaction, which was formerly distinctly observed, was absent; fourth, control experiments, conducted with dead rabbits, showed that the oscillograph record was not changed when the EMF was switched on. Thus, it is apparent that all the above descriptions are of biological reactions.

In order to examine the primary anatomical locus of the EMF effect, we set up experiments involving exclusion of certain analyzers. This revealed that simultaneous section of the optic nerves, exclusion of olfaction, and also destruction of the vestibular apparatus and hearing did not abolish the EMF effect. Consequently, input to the analyzers obviously does not play an essential role in the response to EMF. However, the possibility that other receptor systems are affected is not excluded.

It is very probable that EMF exerts an immediate influence on the brain. The following fact supports this hypothesis. When potentials were led across bone, i.e., when the cranium was not opened, the EEG changes were much weaker and were not always observed. Actually, these experiments do not exclude the possibility that the

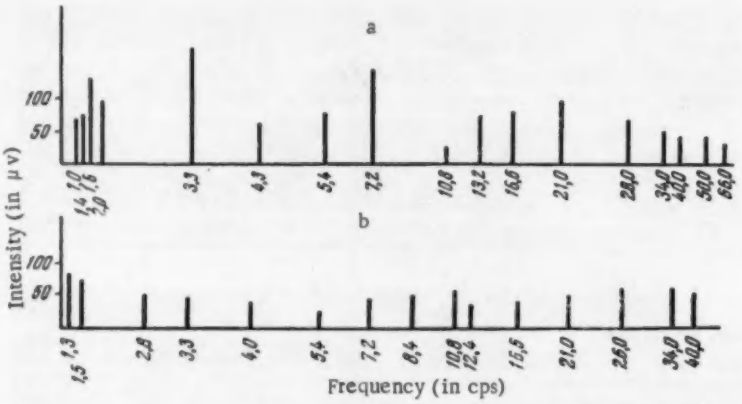

Fig. 2. Analysis of EEG of rabbit exposed to action of EMF. a) Before action; b) during action.

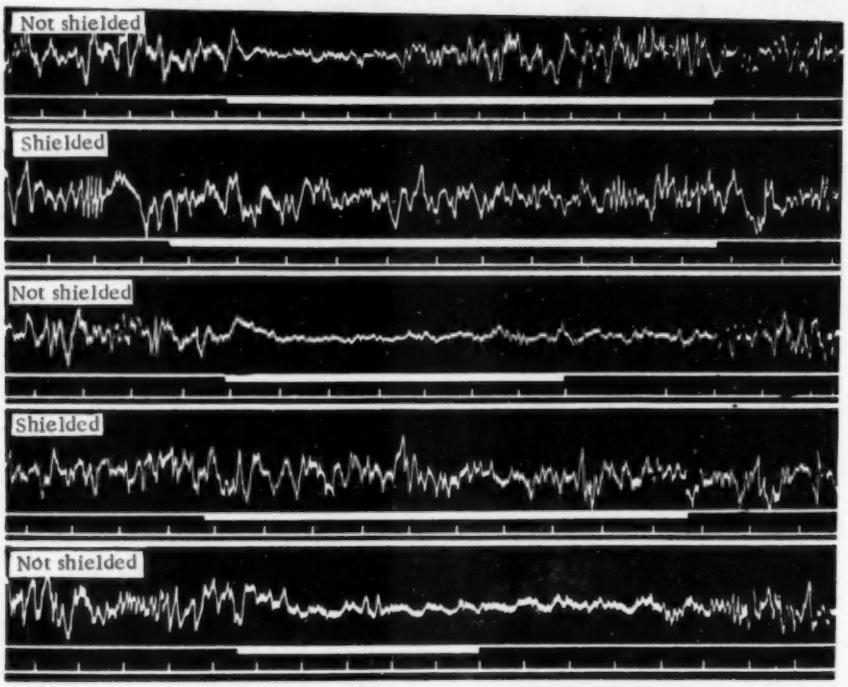

Fig. 3. Influence of shielding the animal on the effect of EMF. Alternate action of EMF with and without shield (rabbit No. 84). Time channels same as in Fig. 1.

increased sensitivity of the brain to EMF results from an augmentation of excitability of neural tissue following trephination.

In addition, it was assumed that the EMF influence on the central nervous system might possibly be due to a direct action of EMF on the recording electrodes; this condition would also cause direct stimulation of the brain. Specifically designed experiments, however, completely excluded this possibility. These experiments, in which the recording electrodes were shunted to ground by condensers of various capacitances (up to 1μ f), showed that under such conditions the effects of EMF were maintained. In addition, it was found that placing the rabbit in a shield consisting of an iron box with walls 3.5 mm thick abolished the observed effect, regardless of the fact that the recording leads passed through and outside the shield (Fig. 3). Consequently, the EMF in our experiments directly affected the animals and altered the activity

of the central nervous system, resulting in distinct changes in the electrical activity of the brain.

SUMMARY

The effect of electromagnetic fields on the bioelectric activity of cerebral cortex was investigated on 98 rabbits. As established, the electromagnetic field provokes signal changes in the electroencephalogram. Increased intensity of the magnetic field raises the reaction of the cerebral cortex. Exclusion of various receptor fields (of hearing, vision, smell, vestibular apparatus) had no effect on the reaction of the brain to the action of the electromagnetic field. This led to a suggestion on the possibility of direct action of electromagnetic fields on the brain.

LITERATURE CITED

1. V. Ya. Danilevskii, Studies on the Physiological Effects of Electric Fields [in Russian] (Kharkov, 1900).
2. L. L. Vasil'ev, Russk. Fiziol. Zhurn. 3, 4 (1921).
3. M. N. Livanov, Trudy Inst. Mozga. Nos. 3-4 (Moscow, 1938), p. 487.
4. F. P. Petrov, Abstracts of Reports of the Fourth All-Union Congress of Physiology [in Russian] (Kharkov, 1930) p. 179.
5. F. P. Petrov, Trudy Inst. Fiziol. im. I. P. Pavlova 1 (Leningrad, 1952), p. 369.
6. E. V. Selivanova and G. M. Erdman, Biofizika 1, 412 (1956).
7. Yu. A. Kholodov, Physiological Analysis of the Effects of Magnetic Fields on Animals (Author's abstract of Master's Thesis) [in Russian] (Moscow, 1958).

MITOGENETIC RADIATION OF THE NEUROMUSCULAR SYSTEM AS A METHOD OF ANALYSIS OF ITS MOLECULAR SUBSTRATE

REPORT I. Analysis of the Interacting Antidromic (Antibathic) Processes and the Degree of their Propagation

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In foregoing publications [1, 2] it was shown that the concepts of A. A. Gurvich on the unequalibrated state of the molecular substrate of living systems—the "physiological" theory of protoplasm— are completely applicable to neuroplasm and myoplasm. Spontaneous radiation of the nervous system and of muscles is regarded from the point of view of a degradation radiation that is physiological in character [2-6].

The structural organization of the unequilibrated, extremely labile substrate is subjected to the constant interaction of the centers and the periphery, among which great importance is held by the influence demonstrated by the muscles on the entire system. This influence, along with sporadic proprioceptive impulsation, manifests itself in the form of continuous activity, spreading centripetally and along motor fibers, i.e., it has an antidromic character [1].

Preliminary data [6] showed that the muscle action permits the genesis of the coarse state in the proteins and peptide chains of the neural fibers, at the same time that the action of the centers can be characterized as a reversing disperser. In other words, regarding the physiological understanding of the interactions of the centers and the periphery, from the points of view of structurochemical relationships, we could speak of the interaction of processes of a reverse—antibathic—character, and we saw our subsequent problem in the study of the connection of these phenomena with the unequilibrated state of the molecular substrate of the nervous system and of muscles.

The present work is related to the further study of the character and degree of propagation of the processes in the nervous system arising under the influence of the muscles.

EXPERIMENTAL METHOD AND RESULTS

The investigation was carried out by studying the mitogenetic radiation of the sciatic nerve and several of its branches in experiments in vivo on frogs. Spectral analysis was used in the series of experiments. In the form of an auxiliary series of experiments, spectral analysis was carried out on the radiation of models containing a well-regulated distribution of protein molecules (running protein solution).

The method of detection of the radiation was based, as in all the foregoing laboratory investigations, on the principle of its biodetection [3, 4, 6].

The determination of intensity of radiation necessary for the clarification of the character of the interacting processes was based on the dependency, studied in special investigations, of the binding intensity of radiation I on the threshold exposure $t(I \cdot t - \text{const}$ [6]).

Determination of the degree of coarsening and dispersion was based on the possibility of estimating the amount of free peptide terminal groups by the intensity of their respective spectral bands [6].

However, for further study of these processes in the substrate of the nervous system, a preliminary study was necessary of this phenomenon on models, comparable in labile regulation of molecules to the unequilibrated state of the substrate in the neural fiber. The simplest model of this type is a slow current of a weak solution of protein in a narrow pipe, connected to a source of molecules of known orientation in regard to the direction of flow. The flowing solution can thus be regarded as a molecularly regulated system of unequilibrated character.

The entire representation in the illustration (Fig. 1) of the system was filled with a weak solution of egg albumin. The wide U-shaped tube was provided with a quartz window. In the low siphon was placed a section of quartz pipe. A closed clamp was used to stop the motion of the solution, and by its use the varying speed of flow was regulated. The left window of the U-shaped tube was irradiated with ultraviolet light from a physical source. The collimator of the quartz spectrograph was centered against the quartz pipe of the lower siphon.

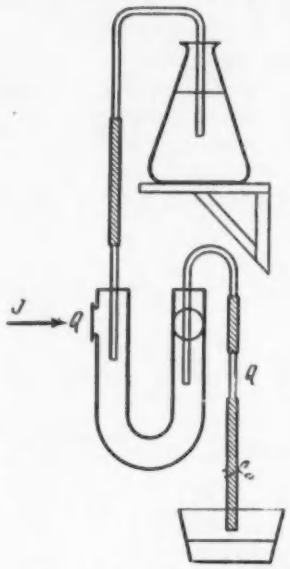

Fig. 1. Scheme of the assembly for the determination in a model of the state of the substrate of a neural fiber. Explanation in the text.

TABLE 1

Radiation Spectra of the Amino Groups in the Protein Solution

Lines of the amino-groups	Solution	
	stationary	flowing
2065–70 Å	27 ± 3.0	0.3 ± 1.58
2260–70 Å	25 ± 3.4 (Average of 8 trials)	4.2 ± 1.81 (Average of 8 trials)

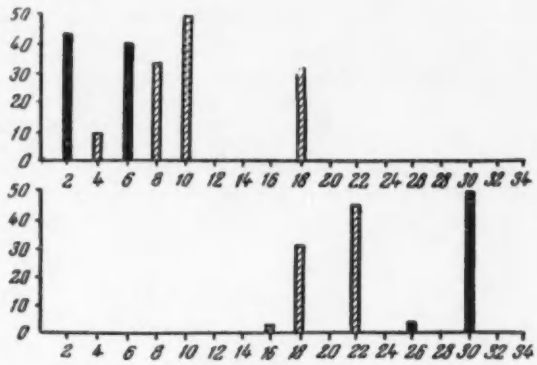

Fig. 2. Distribution of intensity of the radiation of amino groups along the length of the sciatic nerve. Upper series Radiation of spectral lines characterizing the amino group at the proximal end of the nerve; lower, same, at the distal end; crosshatched bars, radiation of the nerve in the resting state; black, the same on stimulation. Exposure is plotted on the abscissae (in seconds); on the ordinates, results (in percent.

Irradiation of the solution with ultraviolet light caused a photochemical chain reaction throughout its entire volume, accompanying the ultraviolet luminescence of mitogenetic intensity with a selective spectrum expressing the molecular composition of the solution [3, 4, 6].

Evaluation of the intensity of the spectral bands, characterizing the terminal peptide groups in the stationary and flowing solutions, showed a greater intensity of radiation, i.e., a larger amount of free terminal groups in the stationary solution in comparison with the flowing solution. In other words, the orientation of the protein chains in the direction of flow already leads to a certain interaction of the terminal groups (the number of free amino groups as though decreasing), which can be observed by the spectral method.

The model experiments thus explained the orientating data obtained by us on the nerve, and permitted making them more precise, thanks to a more detailed spectral analysis of the processes taking place in the unexcited nerve. The frog was placed in front of the spectrograph in such fashion that the proximal or distal portion of the sciatic nerve was centered, in turn, in front of the inlet window. In a comparison of the spectra of these portions a clearly manifested difference in the intensity of radiation was observed; the small exposure necessary for detection of the amino group lines in the proximal end had to be increased by 2–3 times in order to detect the corresponding lines in the distal part. The results were identical by spectral analysis of the radiation at rest and by analysis using the method of selective diffusion (Fig. 2).

Thus, it can be accepted that a continuous activity of the muscles causes a tendency toward coarsening of the peptides in the nerve fibers and that impulses sent by the spinal cord centers counteract this.

It seems probable that the stimulated state, in the presence of which the potential complexity of the system, is in fact realized, would be connected with the clearer manifestation of the processes. Thus, in a series of experiments, the receptor zone of an afferent cutaneous femoral nerve was stimulated with a weak acid solution. The results obtained fortified the hypothesis: exposures in the proximal portion of the nerve were still less than in the resting state; the necessary exposure in the distal portion, on the other hand, lengthened (see Fig. 2).

The results described afforded the possibility of making the general concept on the interaction of anti-

bathic processes more concrete. Its physiological sense may be closer to a complete formulation in the following manner: the "functional" concept of the "connections" of the individual systems with each other entering into a neuromuscular system as a whole should be regarded from the point of view of a distribution of counteracting (and, thus, also interacting) molecular processes.

Significant propagation of interacting processes was already shown in a series of data [1, 3, 6]. The facts presented here are related to the radiation of an afferent cutaneous femoral nerve, and reflect the reaction of that nerve to an action being endured by a segment of the sciatic nerve distant from it.

TABLE 2

Radiation of an Afferent Cutaneous Femoral Nerve (results in %)

Experimental conditions	Before transection of the distal portion of the sciatic nerve	After transection (within a half hour)
Without application of additional stimulation	22, 25, 32, 33*	18, 21, 30, 35
With skin stimulation	N/5 H_2SO_4—25, 22, 24, 40	8, 8, −2, −6
	Pressure on the skin in the region of branching of the nerve (20 g weight) 35, 45	−12, 13
	Pinching of the skin in that region	−3, −15, 9, 4

* Threshold exposure from 15 to 20 sec. Each number represents the results of a single trial.

From Table 2 it is apparent that in "at rest" radiation of an afferent cutaneous femoral nerve transection of the sciatic nerve is not reflected.

The cutaneous nerve radiates in the presence of stimulation, in contrast to the resting state, only with retention of the entire system of the sciatic nerve intact.

The preceding data showed that transection of the sciatic nerve at the distal portion disrupted the regularity (coordination) of the reflex radiations from the semimembranosus and semitendinosus muscles of the thigh in the presence of irritation of the skin in the region of an afferent cutaneous femoral nerve, and, as follows from the data in Table 2, simultaneously disrupts the capacity of the cutaneous nerve to radiate. Definite interest is aroused by the fact that in those comparatively rare instances where the full character of the reflexes was preserved, the capacity of the cutaneous nerve to radiate was likewise preserved [1].

Another reaction of the cutaneous nerve was observed after transection of the motor roots on the same side.

With this latter transection of the roots there appeared a loss of the capacity of the cutaneous nerve to radiate both in the resting state and in the presence of irritation.

In other words, one can state that for the nerve's radiation at the moment of irritation it requires activity on the part of all or almost all of the muscles innervated by the sciatic nerve. For resting radiation the activity of a portion of the muscles is sufficient.

TABLE 3

Radiation of an Afferent Cutaneous Femoral Nerve (results in %)

Experimental conditions	With preservation of the anterior roots intact	After their transection
Without the application of additional stimulation	33, 43, 20, 25,	0, 12, 9, −10
With skin stimulation	40, 33, 25	10, −2

The results presented must be regarded from two points of view. The single fact of the reaction of the cutaneous femoral nerve to disruption of the integrity of a distant nerve trunk clearly shows not only a large degree of spread of muscular activity in the norm, but also its effectiveness in relation to such various elements as synaptic connections, nerve cells, etc.

But the range and complexity of this generally self-propagating reaction are great, and, in the meantime, permit discussing only the following general considerations: Comparatively light disturbance of the regularity of the reflex muscle acts showed that the potency of the regular responses is not connected with a readily accomplishable, i.e., probable, state of the substrate, and that this potency must be regarded as the manifestation of an extremely labile, unequilibrated state. Along with this, only in the presence of this condition is the intensive development of sufficiently energetic chain processes ensured, accompanied by radiation and yielding coordinated reactions. In other words, the "resting" state appears to be the basic "active" background, determining, to a known degree, the character of the uprising deviation—the stimulated state.

SUMMARY

The general conception of the antidromic character of processes on the sciatic nerve is concretized by the method of mitogenetic spectral analysis. Determination of the relative quantities of peptide-free terminal amino-groups demonstrates the prevalence of the prepolymeric processes in the distal portion of the nerve (muscle action)

and of the processes dispersive in character in the proximal area (the action of the centers). Their continuous interaction creates the resulting inequilibrated state of the molecular substrate, also spreading to the afferent paths of the spinal cord system, distant from the muscular connections.

LITERATURE CITED

1. A. A. Gurvich, Byull. Éksp. Biol. i Med. 19, 11 (1945).
2. A. A. Gurvich, Byull. Éksp. Biol. i Med. 19, 3 (1945).
3. A. G. Gurvich and L. D. Gurvich, Mitogenetic Radiation [in Russian] (Moscow, 1945).
4. A. G. Gurvich and L. D. Gurvich, Introduction to the Study of Mitogenesis [in Russian] (Moscow, 1948).
5. V. F. Eremeev, Byull. Eksp. Biol. i Med. 45, 95 (1958).*
6. A. G. Gurvich and L. D. Gurvich, Die mitogenetische Strahlung [Russian translation] (Jena, 1959).

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* Original Russian pagination. See C.B. Translation.

THE INHIBITION OF METHEMOGLOBIN FORMATION BY GLUTAMATE

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We have demonstrated previously [1] that glutamate administered to rats simultaneously with a lethal dose of sodium nitrite prevented the death of the animals and lowered the blood methemoglobin levels by about 40% compared to control values in animals given nitrite alone The effect of glutamate on methemoglobin formation was demonstrated even more clearly in isolated blood in vitro.

However, from the previous experiments with isolated blood it was not clear whether the actual effect of glutamate was to inhibit the nitrite-induced methemoglobin formation, or whether it simply accelerated the reversal of the process—the reduction of methemoglobin to hemoglobin. The present work was undertaken in order to elucidate this fact.

METHODS

White rats were given sodium nitrite subcutaneously, in doses of 100 mg/kg body weight. Blood samples were taken from the tail vein 20 min later for methemoglobin determination, and immediately some of the animals (experimental group) were given sodium glutamate (100 mg per 100 g weight) subcutaneously, while the remaining animals were similarly injected with normal saline. All animals were killed 20 min later by decapitation and blood methemoglobin in all samples was determined by the method of Horn, slightly modified by us [1].

RESULTS

Within 20 min of sodium nitrate administration to the rats, the methemoglobin content of the blood taken from the tail veins of 11 animals corresponded to a conversion of 47.2 % (mean values) of the total hemoglobin to methemoglobin. The results of the assay on the decapitated animals, 20 min after glutamate (experimental animals) or saline (controls) administration, are shown in Table 1.

It will be seen from the Table that in control animals the mean (of 10 experiments) methemoglobin level was 67 %, while in experimental animals the value was 60.5 %, i.e., only 9.7 % lower. It is thus obvious that glutamate, when administered to animals with high blood methemoglobin levels, failed to reduce it back to hemoglobin. Glutamate, apparently, is only capable of inhibition of nitrite-induced methemoglobin formation .

TABLE 1

Blood Methemoglobin Levels in Rats which Received Sodium Glutamate Subsequent to Injection of Nitrite

Experiment No.	Blood methemoglobin, %		Fall in methemoglobin levels in experimentals %
	controls	expt	
1	71,8	66,2	7,7
2	64,0	57,5	11,2
3	74,0	62,2	15,3
4	77,1	67,0	13,3
5	65,7	57,5	12,5
6	59,4	52,4	11,9
7	69,4	62,8	9,8
8	63,7	57,8	9,6
9	61,1	57,0	7,2
10	63,6	63,4	0,5
Mean	67,0	60,5	9,7

It was of interest to observe the protective effect of glutamate when administered prior to the sodium nitrite. Sodium nitrite in these experiments was therefore given to rats 30 min after glutamate injection, and the blood methemoglobin levels were assayed, as before, 20 and 40 min later. Ten rats, given nitrite alone, served as controls. At the two times of assay, methemoglobin levels in experimental (protected) rats were 28.2% and 22% lower, respectively, than in the control rats. Thus, glutamate given 30 min before nitrite administration also exerted an inhibitory effect on methemoglobin formation, although not as efficiently as when administered together with the poison, as was demonstrated earlier [1].

Intravenously introduced glutamate is known [2] to disappear rapidly from the blood stream. It may therefore be assumed that the inhibition of methemoglobin

TABLE 2

The Induction of Methemoglobin Formation in Blood in Vivo by Nitrite in the Presence of Various Amino Acids (exposure time — 2 hr at 18°; each value represents a mean of 8 experiments)

Blood methemoglobin (%) with nitrite and amino acids					
nitrite (control)	glycine	serine	cysteine	leucine	glutamate
85.2	85.6	88.7	87.8	87.1	2.0

TABLE 3

The Effect of Glutamate on Methemoglobin Formation in Isolated Blood Treated with Ferricyanide (exposure— 2 hr at 18°; all values are means of 8 experiment)

Blood methemoglobin (%) after addition of :	
potassium ferricyanide	ferricyanide + + glutamate
70.5	38.7

formation was due to glutamate itself, and not to some product of its catabolism.

It is difficult at present to speculate on the possible mechanism through which glutamate inhibits the nitrite-induced methemoglobin formation. A direct chemical interaction between glutamate and nitrite, resulting in decreased blood levels of the latter, is hardly possible. Although nitrous acid is known to react with amino acids to liberate the amino nitrogen as a free gas, this reaction takes place only in strongly acid environments which can be excluded under in vivo conditions. Should such a reaction be possible, it would be expected to apply in the case of amino acids other than glutamate, as well. This is not actually the case. Under the present experimental conditions, the in vitro addition, to blood treated with sodium nitrite, of corresponding amounts of serine, glycine, cysteine or leucine, had no effect on the nitrite-induced methemoglobin formation, while glutamate, added in the same concentration, almost entirely abolished it (see Table 2).

Glutamate was also found to inhibit considerably the methemoglobin formation in blood treated with ferricyanide (Table 3).

The suppression of methemoglobin therefore appears to be specifically a property of glutamate, which can be demonstrated in the presence of various methemoglobin-producing stimulants.

SUMMARY

Glutamate has been shown to suppress considerably methemoglobin formation in rats in vivo and in isolated rat blood. The mechanism did not involve reduction of methemoglobin to hemoglobin. The effect was specific to glutamate, other amino acids tested (serine, glycine, leucine, cysteine) being ineffective. The protective effect of glutamate also applied to ferricyanide-induced methemoglobin formation. The protective effect of glutamate was maximal when the amino acid was administered simultaneously with the poison.

LITERATURE CITED

1. A. M. Genkin and M. S. Volkov, Byull. Éksp. Biol. i Med. 47, 50 (1959). *
2. V. Klingmüller and K. Z. Vogelgesang, Physiol. Chemie 300, 97 (1955).

* Original Russian pagination. See C.B. Translation.

THE MECHANISM OF NITROGLYCERIN ACTION ON THE CARDIAC VESSELS

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Nitroglycerin is one of the oldest agents used in the treatment of angina pectoris. In the opinion of most clinicians, clinical practice has not yet found any pharmacological substance which acts as quickly and reliably at the time of an angina pectoris attack as nitroglycerin. The efficiency of nitroglycerin is believed to be due to its vasodilative effect on various vascular regions of the organism, particularly the vessels of the heart.

However, examination of the literature on this question shows this seemingly indisputable theory to be based on extremely contradictory data. As early as the beginning of the twentieth century, it was established experimentally that nitroglycerin has a relaxant effect on the smooth musculature of the cardiac vessels [12,14]. This fact was subsequently confirmed at various dates by other researchers experimenting with isolated heart preparations [5, 6, 13].

However, nitroglycerin's vasodilative effect on the cardiac vessels has been shown by experiments to be much less pronounced in the case of an intact organism. A considerable increase in the coronary blood flow (30-100%) under the influence of nitroglycerin has only been noted by authors who used the thermoelectric method of recording the blood flow in their experiments [10, 11]. These observations, however, are not sufficiently conclusive, because it is known that this method cannot be expected to produce quantitatively exact results [8]. Other researchers, who used more exact methods of measuring the coronary output (diaphragm or bubble gauges), found that nitroglycerin and sodium nitrite caused only a slight (7-9 %), brief increase in the volumetric rate of the coronary blood flow when injected intravenously in relatively large doses. The effect of these substances was considerably weaker than that of papaverine or Euphyllin [7, 9].

In our laboratory, I. E. Kisin [2] recently investigated nitroglycerin's effect on the volumetric rate of the outflow of blood from a cat's coronary sinus and on the tonus of the coronary vessels under conditions of the autoperfusion of the latter. He established that nitroglycerin, injected intravenously (0.1-0.5 mg/kg) or administered per os (1-2 mg/kg), does not materially affect the outflow of blood from the coronary vessels or the resistance of the coronary vessels to the blood flow.

It is evident from the literary data cited that the effect of nitroglycerin on the coronary vessels of an intact organism is slight. This leads one to believe that nitroglycerin's efficiency in angina pectoris cannot be explained solely by its vasodilative effect on the coronary vessels.

The fact that nitroglycerin, unlike most pharmacological substances used in the treatment and prophylaxis of coronary insufficiency, is an agent which is effective only to clear up attacks of angina pectoris. Moreover, reflex effects which act on the cardiac vessels and cause them to constrict are known to play an essential part in the development of attacks of angina pectoris.

On the basis of these considerations, we conducted this investigation in order to study the effect of nitroglycerin on reflex changes in the tonus of the coronary vessels.

EXPERIMENTAL METHODS

The experiments were performed on cats anesthetized with urethan (500 mg/kg) and chloralose (40 mg/kg). Because vasomotor reflexes do not affect solely the cardiac vessels, but also cause changes in the arterial pressure, it is difficult to demonstrate these reflexes by measuring the rate of the blood flow in the vessels of the heart. Variations in the blood pressure cause mechanical changes in the blood flow. Artificial stabilization of the blood flow in the coronary vessels, done by perfusing the vessels with the animal's own blood with the aid of a special pump, eliminates the hemodynamic effect of blood pressure changes. Under these conditions, the pressure in the coronary vessels is determined only by the minute volume of the blood supplied by the pump and by the hydraulic resistance of the vessels. Dilatation of the vessels causes the perfusion pressure to fall; their constriction causes it to rise. This method, known as resistography, therefore makes it possible to study the reflex reactions of the coronary vessels in the presence of simultaneous changes in the systemic arterial pressure [3].

The construction of the pump used in the perfusion of the coronary vessels was analogous to that described by V. M. Khayutin, V. M. Danchakov, and V. L. Tsaturov [4]. After opening the thoracic cavity under conditions of artificial respiration, we dissected out the left common coronary artery, placing a special cannula (issuing from the left subclavian artery and connected with the outlet of the perfusion pump) in its mouth. The blood entered the pump from the carotid artery. The perfusion pressure was recorded between the left coronary artery and the outlet valve of the pump. We have already depicted the diagram for experiments with autoperfusion in an earlier work [1]. The blood pressure was recorded in the femoral artery.

Heparin (1000-1500 units per 1 kg) was used to prevent blood coagulation. Nitroglycerin was administered intravenously or per os.

In the experiments, we studied the reflexes on the coronary vessels and the blood pressure induced by stimulation of the carotid sinus receptors (by pressure on the carotid artery) and of the central sections of the femoral and median nerves. The nerves were stimulated with pulses of 2-10 volts from an electronic stimulator 30-50 cps in frequency and 1-2 msec in duration.

EXPERIMENTAL RESULTS

We note first of all that, as in the experiments of other authors, in spite of the considerable fall of blood pressure in our experiments with the administration of nitroglycerin (intravenously in doses of 0.1-0.5 mg/kg or perorally in doses of 1-2 mg/kg), there were no distinctly expressed changes in the tonus of the coronary vessels. In several experiments, moreover, there was even some constriction of the cardiac vessels (Fig. 1).

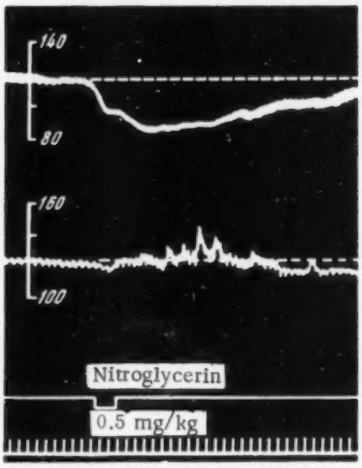

Fig. 1. Effect of nitroglycerin on the resistance of the coronary vessels. Curves (from top to bottom): blood pressure; perfusion pressure (resistogram); administration of substance; time (in 5-second marks). Original levels of blood and perfusion pressures shown by ----- .

We also studied the effect of nitroglycerin on the tonus of the coronary vessels under conditions of experimental spasm of the latter, induced by the intravenous injection of Pituitrin (1.5-2 units per 1kg). In these experiments, nitroglycerin somewhat lowered the resistance of the coronary vessels. Complete elimination of the spasm of the coronary vessels induced by Pituitrin was not observed, even with the administration of nitroglycerin in relatively large doses (0.5-0.7 mg/kg).

Stimulation of the carotid sinus mechanoreceptors and stimulation of the afferent fibers of the tibial and median nerves both produced reflex changes in the tonus of the coronary vessels. In a majority of experiments, pressure on the carotid artery resulted in a reflex constriction of the coronary vessels, although in a few cases, dilatation of the vessels could be observed in response to this stimulation. The same was true of the reflex changes in the tonus of the cardiac vessels produced by stimulating the afferent fibers of the tibial and median nerves.

In doses of 0.1-0.25 mg/kg, nitroglycerin sharply depressed (by 40-80 % of the original level) the reflexes on the coronary vessels in response to stimulation of all the experimental reflexogenic zones. Increasing the nitroglycerin dose to 0.3-0.5 mg/kg completely depressed the reflexes. The effect lasted 15-18 minutes. It is particularly noteworthy that, in most of the experiments conducted, nitroglycerin's effect on the reflex reactions showed a pronounced selectivity with respect to the coronary vessels. When the reflexes on the cardiac vessels were completely suppressed, the reflexes on the blood pressure only decreased an average of 30-50 % of the original level in value (see Fig. 2). In several experiments with the injection of nitroglycerin in doses of 0.1-0.25 mg/kg, the reflexes on the coronary vessels changed in character. Instead of reflex constriction of the vessels, we observed their dilatation (Fig. 3, b). Increasing the nitrglycerin dose to 0.3-0.5 mg/kg led in these cases to complete suppresion of the regional reflexes (Fig. 3 d).

In the experiments with the peroral administration of nitroglycerin (1-2 mg/kg), as in those with its intravenous administration, depression of the reflexes on the coronary vessels was observed. The condition of the vessels themselves, however, was not noticeably changed.

Therefore, the investigations conducted allowed us to establish that nitroglycerin does not cause pronounced dilatation of the vessels of the heart, but does depress their reflex reactions. These data make it imperative to revise the earlier notions concerning nitroglycerin's effect on the cardiac vessels. Obviously, the earlier notion that its efficiency in angina pectoris is due solely to a direct vasodilative effect is invalidated. Our observations, which have demonstrated nitroglycerin's ability to cause selective depression of the reflexes on the coronary vessels, allow the hypothesis that these properties play a significant role in the mechanism of its effect on coronary circulation.

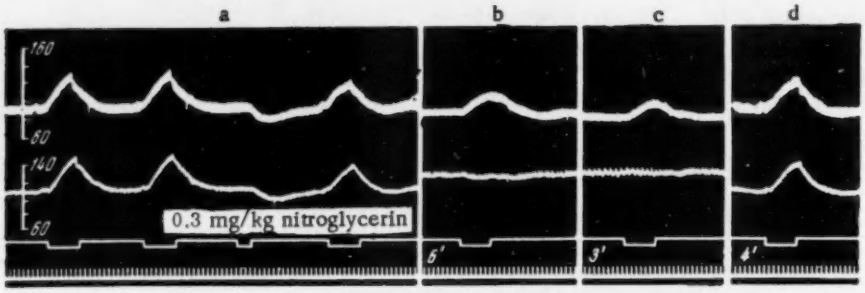

Fig. 2. Effect of nitroglycerin on the reflex changes in the resistance of the cardiac vessels induced by stimulation of the carotid sinus receptors. Symbols the same as in Fig. 1.

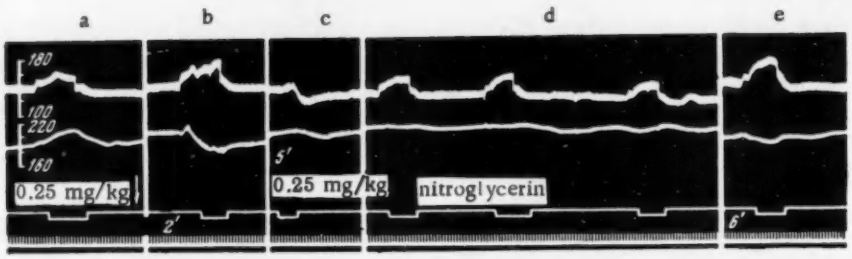

Fig. 3. Effect of nitroglycerin on the reflex changes in the resistance of the cardiac vessels induced by stimulation of the afferent fibers of the tibial nerve. Symbols the same as in Fig. 1.

SUMMARY

The authors studied the effect of nitroglycerine on the reflex resistance changes in the coronary vessels provoked by stimulation of carotid sinus receptors and afferent nerves. While failing to provoke pronounced dilatation of the cardiac vessels, nitroglycerin completely depresses their reflex reactions.

LITERATURE CITED

1. N. V. Kaverina, Byull. Éksp. Biol. i Med. 47, 67 (1959).*
2. I. E. Kisin, The Effect of Certain Pharmacological Substances Used to Treat Angina Pectoris on Coronary Circulation (Dissertation) [in Russian] (1958).
3. V. M. Khayutin, Fiziol. Zhur. 64, 646 (1958).
4. V. M. Khayutin, V. M. Danchakov, and V. L. Tsaturov, Byull. Éksp. Biol. i Med. No. 2, 117 (1958).*
5. Yu. S. Chechulin, Arkh. Patol. No. 4, 40 (1958).
6. R. Bodo, J. Physiol. 64, 365 (1927).
7. N. H. Boyer and H. D. Green, Am. Heart. J. 21, 199 (1941).
8. J. Dorner, Arch. Exp. Path. and Pharm. 221, 312 (1954).
9. J. E. Eckenhoff and J. H. Hafkenschiel, J. Pharm. and Exp. Therap. 91, 362 (1947).
10. H. E. Essex, J. F. Herrick, E. J. Baldes, and F. C. Mann, Am. Journ. Physiol. 117, 271 (1936).
11. H. E. Essex, R. Wegria, J. F. Herrick, and F. C. Mann, Am. Heart J. 19, 554 (1940).
12. C. A. Francois-Franck, Compt. rend. Soc. Biol. 55, 1448 (1903).
13. L. N. Katz, E. Linder, W. Weinstein, D. J. Abramson, and K. Jochim, Arch. int. pharmacodyn. et de therap. 59, 339 (1938).
14. K. Schloss, Deutsches Arch. f. klin. Med. 111, 310 (1913).

* Original Russian pagination. See C.B. translation.

THE EFFECT OF NOVOCAIN ON THE BIOELECTRIC ACTIVITY OF THE BLADDER RECEPTORS

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There have many works studying the action mechanism of Novocain injected into the general blood stream. Novocain's effect on the function of the interoceptors has also interested researchers. The majority of these investigations concern the changes in reflex reactions in response to various stimulations of receptors under the influence of Novocain. Only recently have works appeared which are directly concerned with the effect of Novocain on the receptors. These investigations used the electrophysiological method to record the bioelectric activity of vascular receptors from humorally isolated veins [6, 7] or of organic interoceptors after the direct application of Novocain to the receptors [5]. We know of only one work [10], in which the electrophysiological method was used to study the effect of Novocain on the lungs and heart after the introduction of the preparation into the general blood stream.

Our purpose was to study the change in the bioelectric activity of the bladder interoceptors caused by the introduction of Novocain into the general blood stream.

The bioelectric activity of the bladder receptors has been thoroughly studied. It has been established that there are no afferent impulses from the bladder receptors when the bladder is empty. Impulsation is increased at the time of the introduction into the bladder or the excretion from the bladder of a physiological solution, i.e., when there is a rapid rise or fall of the intravesical pressure. Periodic changes in impulsation can also occur as a result of changes in the condition of the vesical musculature. Continuous maintenance of a specific intravesical pressure is attended by adaptation of the receptors and by the disappearance of impulsation. The higher the pressure, the slower the adaptation of the receptors. Adaptation to low pressure develops more rapidly [1, 2, 11, 12].

EXPERIMENTAL METHOD

The experiments were performed on cats under ether-urethan anesthesia. Initial etherrausch anesthesia was deepened by an intramuscular injection of a 20% urethan solution in a dose of 1 g per 1 kg of animal weight.

The abdominal cavity was opened along the linea alba of the abdomen, and the bladder was brought out.

The afferent ramulus of the pelvic nerve on the lateral surface of the bladder was ligated and sectioned, after which the peripheral end was placed upon an electrode. The impulses from the nerve were recorded on a two-channel OB-2 cathode-ray oscillograph. By means of a catheter inserted into the urethral canal, the bladder was evacuated of urine and filled with 10-20 ml of a warmed Ringer's solution in order to distend the bladder walls to a uniform and constant degree. During the experiment, the bladder was kept covered with a cloth moistened with warm Ringer's solution. A 1 % solution of Novocain was introduced into the femoral vein in a dose of 10 mg/kg.

EXPERIMENTAL RESULTS

The results produced by the 14 experiments performed were uniform in type. We noted a lack of definite rhythmicity in the impulses recorded from the vesical interoceptors.

Impulses of varying amplitude developed non-rhythmically; as other authors also have observed, the original activity of the bladder receptors depended on the degree to which the bladder was filled. The degree of the original activity usually did not affect the reaction of the receptors to the Novocain injection. Using the Ringer's solution to fill the bladder to a uniform degree, we could record the distinct changes caused by the intravenous injection of Novocain.

In 9 out of 14 experiments, Novocain was observed to have a phasic effect on the bladder receptors; the activity first became intensified, then depressed, and subsequently, restored. The phasic effect of Novocain on the receptors was well expressed in the experiment described in Fig. 1. In this experiment, the initial activity (a) was recorded after introduction of 10 ml of Ringer's solution into the bladder. The novocain was administered over a period of 1 min 35 sec. Forty seconds after the injection ceased (b), the activity increased. The impulsation decreased after 1 min 50 sec (c), and, after 6 min 13 sec (d), the impulses became less frequent. After 7 min 20 sec (e), however, acceleration occurred, and towards the end of this segment of the recorded oscillogram, the impulsation had returned to the original level. Ten minutes after

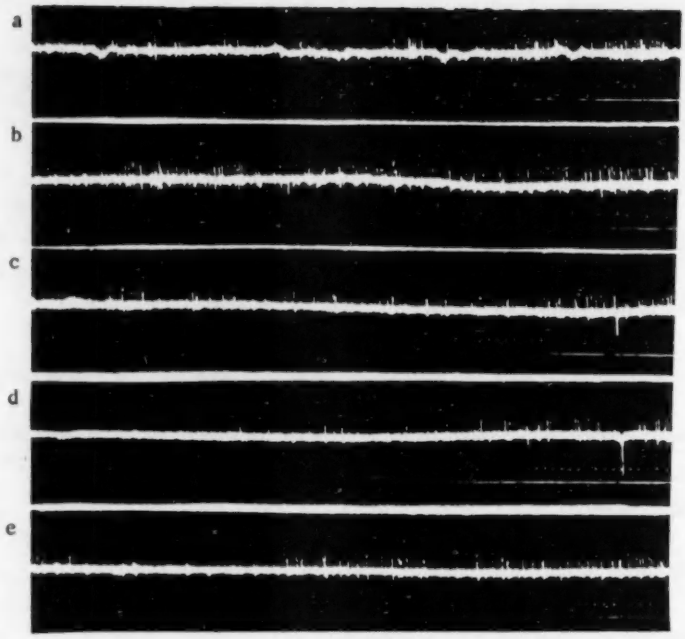

Fig. 1. Effect of novocain on impulsation from the bladder receptors. a) Before novocain injection; b) 40 seconds after novocain injection (10 mg/kg) ceased; c) 1 min 50 sec after injection; d) after 6 min 13 sec; e) 10 min 10 sec after novocain injection.

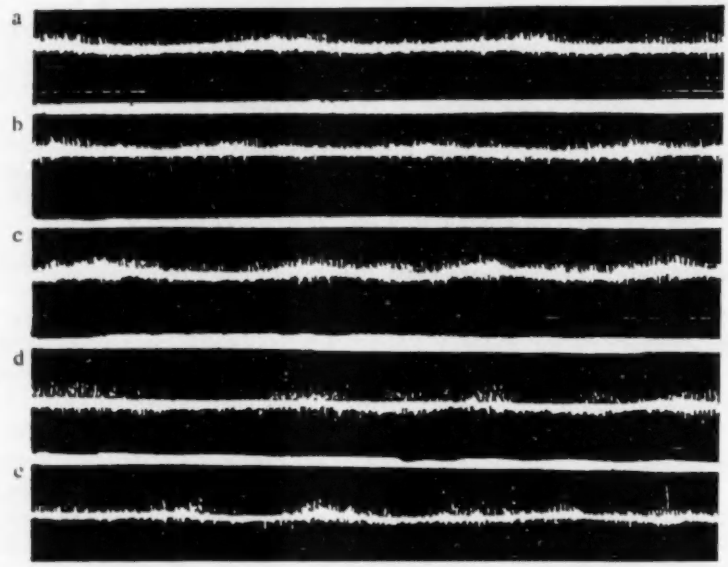

Fig. 2. Effect of novocain on impulsation from the bladder receptors. a) Before novocain injection; b) immediately after injection of 10 mg/kg novocain; c) 2 min 30 sec after novocain injection ceased; d) after 15 min; e) 18 min after injection.

the Novocain injection ceased, the impulses from the bladder interoceptors were stabilized at the original level.

Several other works [5, 7, 8, 9] mention the phasic effect of Novocain. In our previous investigations studying Novocain's effect on the bioelectric activity of the venous receptors, the tactile receptors of the skin, and the pressoreceptors of the arch of the aorta [3, 4], we also observed a biphasic change in the impulsation.

Along with the phasic changes which occurred in the above 9 experiments, in 3 experiments we observed only a brief intensification of the impulsation from the bladder receptors, and in 2 experiments we observed primary depression of the latter. Intensification of impulsation, or exaltation, was well expressed in the experiment described in Fig. 2. Impulsation began to increase immediately after the Novocain injection. It reached its greatest intensity after 2.5 min (c), remained at the high level for a rather long time until, after 18 min, it decreased to the original level without any apparent subsequent depression. In the following experiment (Fig. 3), primary depression of the impulsation from the bladder receptors occurred. Forty seconds after the Novocain injection began (b), the activity started to decrease. It was still depressed after 6 min 17 sec (c), but the impulses returned to the original frequency and amplitude after 11 min 10 sec (d).

A characteristic of all the experiments was the brevity of the reaction of the bladder interoceptors. Both the changes in the activity and its restoration to the original level developed very quickly regardless of the type of reaction—depression or exaltation. The maximal duration of the interoceptor reaction to the Novocain injection was 18 min, but usually the reaction lasted only 5-10 min.

Zipf [10], who studied the action currents of the afferent nerves of the heart and lungs before and after the administration of various anesthetic substances, including Novocain, described similar changes in impulsation from the interoceptors. He administered Novocain once intravenously and also in the form of a prolonged transfusion and observed that the afferent impulses traveling from the lungs and heart only disappeared after the prolonged infusion. This depression, however, was brief, and the number of impulses returned to the original level as early as 10-15 min after the infusion. After the single intravenous injection of Novocain, impulsation from the receptors decreased only slightly and did not disappear altogether.

SUMMARY

The author studied the effect of the intravenous injection of Novocain on the impulse activity of the cat's bladder receptors. The impulses were recorded in the peripheral end of the afferent branch of the

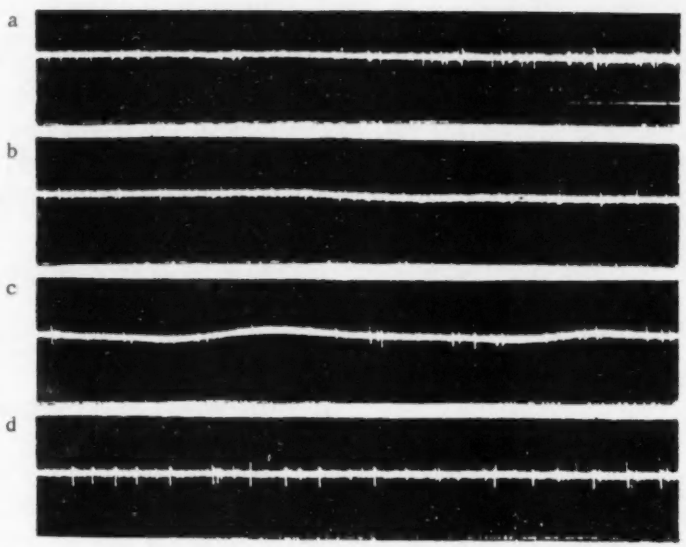

Fig. 3. Effect of novocain on impulsation from the bladder receptors. a) Before novocain injection; b) 40th second of novocain injection (10 mg/kg); c) after 6 min 17 sec; d) 11 min 10 sec after novocain injection.

pelvic nerve. A 1% Novocain solution was injected into the femoral vein in a dose of 10 mg/kg.

The investigations show that a single intravenous injection of Novocain does not block the bladder interoceptors. The brief (5 to 18 minutes) reaction of these receptors to the administration of Novocain was usually manifested by an initial increase in the activity and its subsequent inhibition. Occasionally, however, a brief incomplete inhibition of the intensification of the receptor activity was noted.

LITERATURE CITED

1. N. A. Adamovich, Transactions of the I. P. Pavlov Institute of Physiology 3 [in Russian] (Moscow-Leningrad, 1954) p. 490.
2. V. G. Boksha, Fiziol. Zhur. 43, 1149(1947).
3. N. F. Glebova, Abstracts of Reports at the Scientific Conference of the Physiologists, Biochemists and Pharmacologists of the Western Siberia Society, Commemorating the 40th Anniversary of the Great October Socialist Revolution [in Russian] (Tomsk, 1957) p. 66.
4. N. F. Glebova, Proceedings of the Far-Eastern Branch of the Society of Physiologists, Biochemists, and Pharmacologists [in Russian] (Khabarovsk, 1958) p. 8.
5. M. V. Kirzon, O. R. Kol's, and A. M. Tsukerman Problems of Clinical and Experimental Surgery [in Russian] (Moscow, 1953) No. 2, p. 82.
6. O. P. Minut-Sorokhtina, The Role of the Venous Thermoreceptors in the Regulation of Heat Metabolism (Author's Abstract of Dissertation) [in Russian] (Leningrad, 1953).
7. O. P. Minut-Sorokhtina and B. Z. Sirotin, The Physiological Importance of the Venous Receptors [in Russian] (Moscow, 1957).
8. M. R. Mogendovich, The Reflex Interaction of the Locomotor and Visceral Systems [in Russian] (Leningrad, 1957).
9. A. K. Chuvaev, Doklady Akad. Nauk. SSSR 63, 601 (1948).
10. H. F. Zipf, Arch. exper. Path. und Pharmakol. 318, 113 (1953).
11. M. Talaat, J. Physiol. 89, 1 (1937).
12. M. Stella, J. Physiol. 82, 22 (1934).

THE USE OF CYSTEINE TO NORMALIZE INTEROCEPTIVE REFLEXES ALTERED BY THE ACTION OF TOXIC SUBSTANCES OF ASCARIDES*

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In articles published earlier [1, 2, 3], we showed that the action of toxic substances of ascarides on the intestinal chemoreceptors causes change in the excitability of the afferent nerve endings. As a result, the vascular and respiratory reflexes from these receptors in response to the customary stimuli (acid, alkali, acetylcholine) are replaced by biphasic pressor-depressor, or solely depressor, reactions on the part of the blood pressure and by inhibition on the part of the respiration. The changes in the reflexes were rather stable; prolonged perfusion with Locke-Ringer's solution for more than two hours did not restore the normal reactions.

We set ourselves the task of finding agents which would normalize reflexes disturbed under conditions of ascariasis. On the basis of the literary data and on the material from our own investigations, cysteine was used under different experimental conditions for this purpose. The experimental results are presented in this article.

EXPERIMENTAL METHOD

The experiments were performed on cats under intravenous urethan anesthesia (a total of 49 experiments were performed). We studied the vascular and respiratory reflexes from the chemoreceptors of a humorally isolated intestine before and after the action of ascarides toxic substances and then after the use of a solution of cysteine. We used acetic acid (0.5-1 ml of a 1% solution), sodium bicarbonate (1-2 ml of a 1.5 % solution) and acetylcholine (0.5-1 ml of a $1 \cdot 10^{-4}$ solution) to stimulate the receptors. As material containing toxic substances of the ascaris, we used water extracts of Ascaris suum, prepared according to E. N. Pavlovskii's method, in part of the experiments and the products of the vital activity of Ascaris suum, obtained by the usual method, in the rest of the experiments. We took into account the fact that the water extracts of the parasites and their metabolites produced in vitro which we used in the experiments were not in themselves equivalent to the helminthotoxins. However, the extracts are assumed to correspond to a specific degree to the endotoxins released in the intestine of the host upon its digestion of the parasite's body, while the products of their vital activity in vitro are assumed to correspond to the exotoxins of the helminths. V. N. Chernigovskii's well-known method was used to isolate the small intestine.

EXPERIMENTAL RESULTS

Since ascarides are usually localized in the small intestine, in the first series of experiments, we introduced the material containing the toxic substances of the parasites into the lumen of the isolated intestine. Under the influence of the helminthotoxins, the reflexes ordinarily elicited by our experimental stimulants (acid, alkali, acetylcholine), i.e., increased arterial pressure and respiratory stimulation, were supplanted by biphasic pressor-depressor or solely depressor blood pressure reactions, and , often, by respiratory inhibition . It should be mentioned that the larger doses of the toxic material caused the greatest changes in chemoreception . We had previously [1] established thar prolonged perfusion with a Locke-Ringer's solution for over two hours does not restore the normal reflexes. To normalize chemoreception in these experiments, we used a solution of cysteine, which we introduced into the vascular system of the isolated intestine through the perfusion course 40-60 min after the administration of the helminthotoxins. It was found that cysteine restored the normal vascular and respiratory reflexes in a large majority of cases.

It is known that interoception can be studied by another method, in which the active principle is supplied to the nerve endings through the vascular system of a humorally isolated organ. The reactions observed with the use of this method should be regarded as the sum total of the reactions from the vascular receptors and the tissue elements of the actual organs. Another experimental series was performed according to this method: The ascarides toxic material was introduced into the vessels of the isolated intestine, and chemoreception was examined before and after the action of the helmin-

* Presented at the meeting of the Visiting Session of the Department of Sciences of the Academy of Sciences of the Byelorussian Soviet Republic, December, 1958 (Vitebsk).

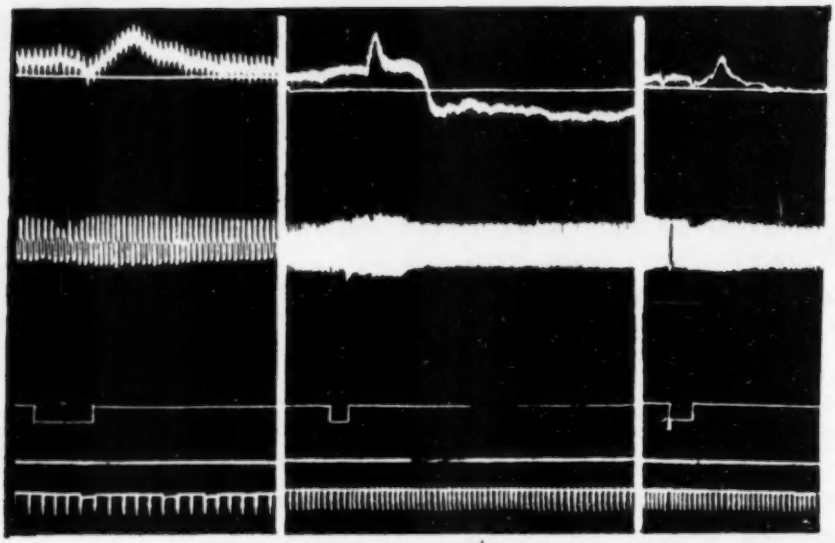

Fig. 1. Vascular and respiratory reflexes from intestinal chemoreceptors to 1 ml of a 2% $NaHCO_3$ solution before (a) and after (b) the influence of ascarides toxic substances and after the use of cysteine (c). Experiment Dec. 14, 1957. Curves show (from top to bottom): blood pressure (mercury manometer); original blood pressure level; respiration; indication of stimulation; zero level of manometer; time (in 5 sec marks).

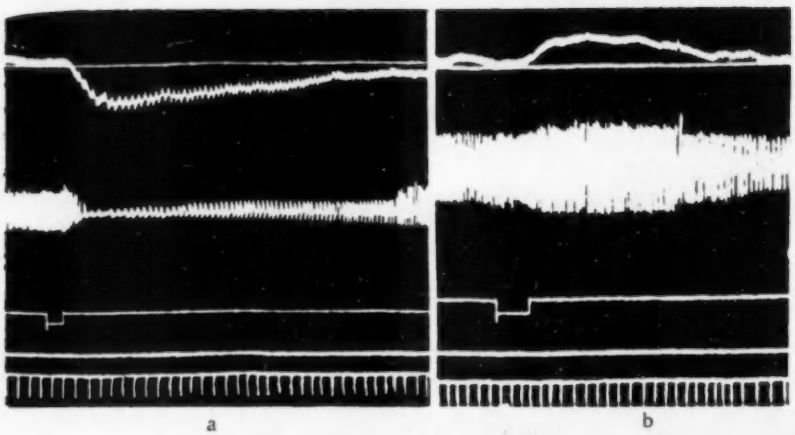

Fig. 2. Vascular and respiratory reflexes from the intestinal chemoreceptors of a cat to 1.5 ml of a 1.5% $NaHCO_3$ solution with natural ascariasis (a) and after the use of cysteine (b). Experiment Jan. 13, 1959. Curves the same as in Fig. 1.

thotoxins and the use of cysteine. The results of these experiments were essentially the same as those of the first series of experiments. In the second series, as in the first, the character of the vascular and respiratory reflexes changed under the influence of the helminthotoxins. Normalization of the reactions by cysteine succeeded to the same degree.

Analysis of all the experimental results shows that, regardless of the type of toxic material used (extract or products of the vital activity), the contact of the intestinal chemoreceptors with these ascarides toxic products was attended by the disturbance of chemoreception and the distortion of the vascular and respiratory reflexes to the usual stimulants. The use of cysteine, however,

Vascular and Respiratory Reflexes from Intestinal Chemoreceptors before and after the Influence of Ascarides Toxic Substances and then after the Use of Cysteine

Expt. date	Experimental conditions	Time (in hours and minutes)	Stimulant and dose	Change in arterial pressure*(in millimeters of mercury)	Reaction duration (in seconds)	Change in respiration
Dec. 14, 1957	Before administration of ascarides toxic material	11.57	1 ml of 2% $NaHCO_3$ solution	+ 14	45	Mild stimulation
	After administration of ascarides toxic material	12.59	The same	+ 14; − 14	290	Mild inhibition
	After administration of cysteine . . .	13.50	The same	+ 12	40	Mild stimulation
Sept. 19, 1958	Before administration of ascarides toxic material	11.23	1 ml of 1% acetic acid solution	+ 4	20	None
	After administration of ascarides toxic material	12.25	The same	+ 10; − 2	37	None
		12.29	The same	+ 4 ; − 2	155	None
		12.59	The same	+6 ; −6	145	None
	After administration of cysteine	13.07	The same	+ 2	15	None
Sept. 19, 1958	Before administration of ascarides toxic material	11.59	1 ml of 1 10^{-4} acetyl-choline	+8	110	Mild stimulation
	After administration of ascarides toxic material	12.49	The same	+ 14; − 36	295	Stimulation—acute inhibition
	After administration of cysteine	13.21	The same	+ 5	40	Mild stimulation
Jan. 20, 1959	With natural ascariasis	11.40	1 ml of $1 \cdot 10^{-4}$ acetyl-choline	+ 2; − 30	Not restored after 20 minutes	None
	After administration of cysteine	12.52	The same	− 3	50	Mild stimulation
		13.39	The same	+ 3	25	Inhibition

* Plus = rise, minus = fall of arterial pressure.

caused the stable normalization of chemoreception disturbed under the influence of experimental ascariasis. One can therefore propose that blockade by the toxins of the sulfhydryl groups of the active protein complexes, or enzymes, plays an essential role in the action mechanism of ascarides toxic substances. This process is reversible; saturation by substances rich in SH groups (cysteine) effects the restoration of the former reflexes, i.e., normalizes the reflexes (Fig. 1).

Our experiments studying chermoreception under conditions of natural ascariasis were of particular interest. During the acute experiments with humoral isolation of the small intestine, we occasionally discovered natural ascariasis in the cats. This material was of course extremely useful to our research. First of all, we established that, with pronounced ascariasis, chemoreception was altered in a way similar to that which we observed with experimental ascariasis intoxication, i.e. the vascular and respiratory reflexes from the chemoreceptors of the isolated intestine in response to the customary stimulants (acid, alkali and acetylcholine) were distorted, the reactions of the arterial blood pressure becoming pressor-depressor or solely depressor and the respiration usually becoming inhibited. After establishing these changes in chemoreception, we used the cysteine solution to normalize the reflexes. The experiments showed that chemoreception altered under the influence of natural ascariasis can be normalized with the aid of cysteine (Fig. 2). The results of the experiments examining chemoreception in cats with natural ascariasis confirmed the changes we established in the character and duration of interoceptive reflexes under the influence of ascarides toxic substances. The theory that blockade by these toxins of the sulfhydryl groups plays an important part in the action mechanism of ascarotoxins was also confirmed.

The data from 4 experiments are included by way of illustration (see table).

Therefore, the changes in chemoreception caused by ascariasis are evidently connected with biochemical shifts in the receptor field which occur under these conditions. This was also indicated to a certain degree by the change we established in special investigations (14 experiments) in the pH of the perfusate flowing out of the vein of the intestine after the action of the ascarides toxic substances. In these experiments, we usually observed the concentration of hydrogen ions in the perfusate to increase considerably, up to as much as pH = 0.46; in the control experiments, however, the maximal change in the pH during the experiment was 0.06.

Our next project was to determine whether cysteine had a detoxicating effect under conditions of general ascariasis. We conducted acute experiments with the use of cysteine under conditions of experimental ascariasis induced by administering the toxic material into a non-isolated intestine or into the animal's general blood stream. These investigations established that intravenous injections of the cysteine solution decreased or completely prevented disturbances in circulation and respiration caused by the action of ascarides toxic substances. These investigations will be continued under conditions of chronic experiments.

SUMMARY

In acute experiments on cats under urethane anesthesia the author studied the change of the vascular and respiratory reflexes from the chemoceptors of a humorally isolated intestine following the action of ascarides toxic substances (Ascaris suum water extracts or the metabolites of these parasites produced in vitro), as well as the normalizing effect of cysteine. As established, the vascular and respiratory reflexes, altered as a result of the toxic products of ascarides, become normalized after the administration of cysteine. In the presence of marked ascariasis in cats, the interoceptive reflexes from the chemoceptors of the isolated intestine are similarly changed; cysteine administration also normalizes the reflexes in natural Ascaris infestation.

LITERATURE CITED

1. I. A. Edel'shtein, Byull. Éksp. Biol. i Med. No. 1 (Supplement), 111 (1957).
2. I. A. Edel'shtein, Byull. Éksp. Biol. i Med. 47, 65 (1959).
3. I. A. Edel'shtein, Med. Parazitol. No. 4, 408 (1958).

† Original Russian pagination. See C. B. translation.

A BASIS FOR CRITERIA OF SENSITIVITY, RESISTANCE AND TOLERANCE IN RELATION TO THE EFFECT OF CERTAIN PHARMACOLOGICAL SUBSTANCES AT DIFFERENT AGE PERIODS

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(Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

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The concepts of sensitivity, resistance or tolerance are often used to compare the reactions of animals of different ages to the effect of pharmacological substances. These concepts are used indiscriminately, and the same index is usually used as the criterion, i.e., the difference in the values of the lethal doses [16 -21 and many others]. In many works, the data showing the percentage of lethality with the administration of pharmacological substances in doses ranging from the maximum tolerance to the absolute lethal are first processed by the method of cumulative frequencies, then expressed graphically and defined as curves of individual sensitivity [5, 7, 8, 9, 10 et al.]. I. A. Arshavskii's definition [1, 2, 3] of resistance as the ability of the organism to maintain a normal or even somewhat increased functional level due to the increase of true accommodative resistance to the active stimulant is based on research investigating the lability of the centers regulating the activity of the respiratory, cardiovascular and skeletal muscular systems under conditions of alteration in animals of different ages. This reaction enables the organism to preserve and sustain the condition usually known as homeostasis. The criterion of resistance used in our laboratory is the duration and degree of expression of the first phase of the parabiotic reaction.

In order to further develop and crystallize the concepts and criteria of sensitivity, resistance and tolerance to the action of pharmacological substances, we made a physiological analysis of the reactions of animals of different ages to the administration of certain pharmacological substances. These investigations enabled us primarily to differentiate between the concepts of sensitivity and resistance.

In the physiological sense, the concept of sensitivity is close to the notion of excitability, or the stimulation threshold. The minimal dose causing certain formations of the organism to react in a fashion specific to the given substance without eliciting a reaction from other formations should be regarded as the criterion of sensitivity to the action of pharmacological substances [13-15].

In the case of most pharmacological substances, there is great difference between the values of the threshold and lethal doses. The latitude between the minimum effective and the lethal doses of many pharmacological substances is completely different in the case of very young animals from that observed for adult animals. For example, the threshold dose of atropine blocking the cholinoreceptive substance of the heart for very young puppies is 1/500 of the same dose for adult dogs, while the minimum lethal dose for the former is only 1/2 of that for the latter [12]. However, the usually accepted method of comparing sensitivity according to the range of the lethal doses does not take these differences into account.

EXPERIMENTAL METHOD

In order to obtain more precise criteria of resistance and tolerance, we performed experiments on baby rabbits 3-8 days old and on adult rabbits. Chloral hydrate was injected intraperitoneally in doses of 5-800 mg/kg, each dose being administered to a group of 3-11 rabbits of the same age (except the doses of 5 and 10 mg/kg in the case of the adult rabbits). The rabbits for the experimental groups were selected not only according to age and weight, but also according to physiological conditions as determined by temperature and by electrocardiogram. The original temperature of the baby rabbits used in the experiments ranged from 35 to 38°, the cardiac rhythm, from 240 to 315 per minute. The animals' reactions were evaluated according to the electrocardiogram and from the changes in respiration and temperature.

EXPERIMENTAL RESULTS

A resistance type of reaction in a rabbit 3 days old, i.e., when the centers which regulate the activity of the respiratory and cardiovascular systems, as well as the centers of thermoregulation, maintain the original or an increased level of activity after the administration of 125 mg/kg chloral hydrate, is shown by the curves in Fig. 1 above the line representing the original level. The

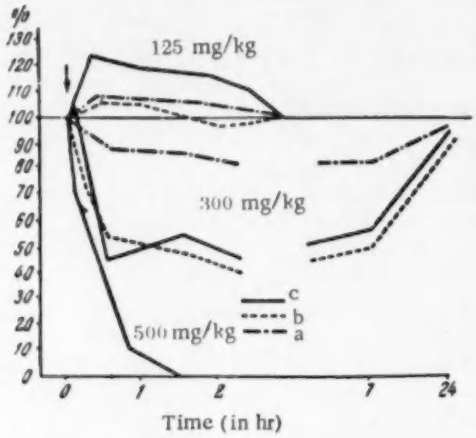

Fig. 1. Graph showing the changes in temperature (a), respiratory rhythm (b) and rhythm of heart contractions (c) characterizing the resistance type of reaction in rabbits aged 3-8 days to the administration of 125 mg/kg chloral hydrate (shown above line representing original level, taken as 100%). Below the line of the original level are shown the same indices for the tolerance type of reaction to the administration of 300 mg/kg chloral hydrate. The lowest curve represents the changes in the heart rhythm caused by the administration of a lethal dose of chloral hydrate (500 mg/kg).

curves below the original level are those showing changes in the temperature and in the cardiac and respiratory rhythms of 5-day old rabbits after the administration of chloral hydrate in doses 300 and 500 mg/kg, which caused the lability of these systems to decrease. The latter reactions represent the protracted collapse typical of a very young organism, during which the nerve centers drop to a lower functional level under conditions of decreased body temperature [6, 11, 14].

With a dose of 300 mg/kg chloral hydrate, the collapse is reversible. This type of reaction indicates a condition of tolerance in the organism, which is to be understood as a reversible disturbance of homeostasis under conditions of intoxication.

Figure 2 gives electrocardiograms taken from baby rabbits (3 and 5 days old) showing reactions of the resistance (a) and tolerance (b) types and the reaction to the administration of a lethal dose (c).

The analysis conducted made it possible to establish that the range of the chloral hydrate doses under the influence of which the reaction characterized by maintenance of homeostasis (first phase) occurs in baby rabbits is very different from the analogous range for adult rabbits. The range of the doses causing the reversible second phase of the reaction and the range of the lethal doses also differ considerably in these two age groups (Table 1).

The absolute lethal dose of chloral hydrate for the baby rabbits was considerably smaller than that for the adult rabbits. A similar correlation between the lethal

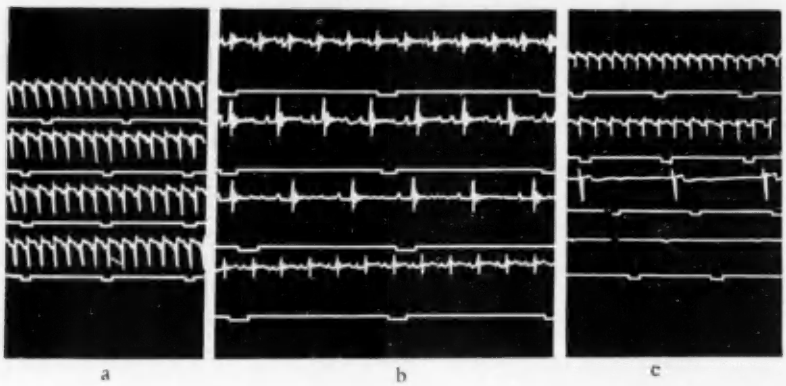

Fig. 2. Electrocardiograms of rabbits. a) 3 Days old before (top line) and 15 min, 1 hr 15 min and 2 hr 30 min after the administration of 125 mg/kg chloral hydrate (second, third, and fourth lines from the top, respectively); b) 5 days old before (top line) and 1 hr, 2 hr, and 24 hr after the administration of 300 mg/kg chloral hydrate (second, third and fourth lines from the top, respectively); c) 5 days old before (top line) and 20, 50, and 60 min after the administration of 500 mg/kg chloral hydrate (second, third, and fourth lines from the top, respectively). Time mark = 1 sec.

TABLE 1. The Different Ranges of Chloral Hydrate Doses Causing the First and Second Reaction Phases in Rabbits of Different Ages

Doses	Rabbits	
	3-8 days old	adult
	chloral hydrate doses (in mg/kg)	
Causing first reaction phase in all experimental animals	5—125	5—450
Producing variation of first reaction phase and reversible second phase	150—250	500—700
Causing reversible second phase of reaction in all animals	275—300	—
Causing variation of second reaction phase-reversible and irreversible	350—450	650—750
Absolute lethal	500	800

doses for rabbits of these two age periods has also been observed in the case of morphine [19, 20].

Table 2 gives the complete data on the baby and adult rabbits, expressed in absolute, integrated (according to Berens) and percentile terms .

On the basis of the data in Table 2, we constructed in Fig. 3 the curve of variation for the percent of death in baby rabbits (B_1) administered chloral hydrate in doses ranging from the maximum tolerance (300 mg/kg) to the absolute lethal (500 mg/kg) and an analogous curve for adult rabbits (B) given doses ranging from 600 to 800 mg/kg. Curves A_1 and A show the variation in the resistance and tolerance types of reactions in baby rabbits (A_1) and adult rabbits (A) given doses within the ranges specified in Table 1.

Left of curve A_1 in Fig. 3 lies the dosage range of 5-275 mg/kg, which includes the doses to which all or some of the baby rabbits of each experimental group reacted by increased mobility of the nerve centers. We call this the range of resistance. The range of tolerance, or the doses ranging from 150 to 450 mg/kg, to which all or some of the rabbits of the experimental groups reacted by a reversible transition to the second reaction phase, lies between the curves A_1 and B_1. The range of resistance for the adult rabbits is shown by the horizontal shading left of curve A (5-700 mg/kg); the vertical shading between curves A and B represents the range of tolerance, and the diagonal shading to the right of curve B shows the range of lethal doses.

Curves A and A_1 cannot be said to depict all cases of the resistance type of reaction, because this reaction was observed in the baby rabbits with the administration of doses less than 125 mg/kg, and in adult rabbits, with doses less than 450 mg/kg, beginning with a dose of 5 mg/kg.

It is the ranges of resistance, which differ for each age group, rather than the curves A and A_1 which give the most complete picture of resistance, just as tolerance is best characterized by the range of tolerance for each age group as well as by the curves B and B_1. Curves A and A_1 are both the right margin of the ranges of resistance and the left margin of the ranges of tolerance; the

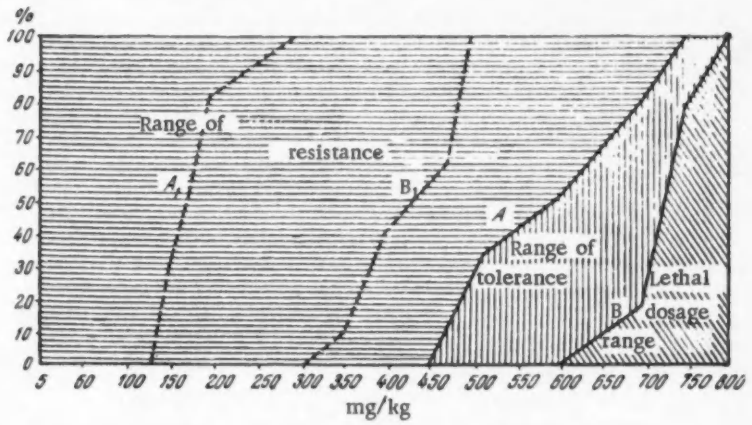

Fig. 3. Variation curve of resistance-tolerance (A) and variation curve of tolerance lethality (B) for adult rabbits. Horizontal shading) range of resistance for adult rabbits. The dotted lines drawn inside the range of resistance of the adult rabbits are the variation curve of resistance-tolerance (A_1) and the variation curve of tolerance-lethality (B_1) for baby rabbits. The shading and notations on Fig. 3 concern the adult rabbits.

TABLE 2. Variation of Resistance and Tolerance Reactions and Lethal Issues in Rabbits with the Administration of Chloral Hydrate

Dose (in mg/kg)	Total No. rabbits	Reaction phases: in absolute nos. first	in absolute nos. 2nd.	in integrated form first	in integrated form 2nd.	in percent first	in percent 2nd.	Lethality of rabbits: in absolute numbers alive	in absolute numbers dead	in integrated form alive	in integrated form dead	in percent alive	in percent dead
A. Rabbits 3–8 days old													
5	4	4	0	25	0	—	—	4	0	—	—	—	—
10	3	3	0	21	0	—	—	3	0	—	—	—	—
50	3	3	0	18	0	—	—	3	0	—	—	—	—
100	4	4	0	15	0	—	—	4	0	—	—	—	—
125	4	4	0	11	0	100,0	0	4	0	—	—	—	—
150	5	2	3	7	3	70,0	30,0	5	0	—	—	—	—
175	5	3	2	5	5	50,0	50,0	5	0	—	—	—	—
200	6	1	5	2	10	16,7	83,3	6	0	—	—	—	—
250	4	1	3	1	13	7,1	92,9	4	0	—	—	—	—
300	6	0	6	0	19	0	100	6	0	23	0	100,0	0
350	9	0	9	0	28	—	—	7	2	17	2	89,5	10,5
375	3	0	3	0	31	—	—	2	1	10	3	76,9	23,1
400	6	0	6	0	37	—	—	3	3	8	6	57,2	42,8
450	11	0	11	0	48	—	—	5	6	5	12	29,4	60,6
500	6	0	6	0	54	—	—	0	6	0	18	0	100,0
B. Adult rabbits													
5	2	2	0	31	0	—	—	2	—	—	—	—	—
10	2	2	0	29	0	—	—	2	—	—	—	—	—
50	3	3	0	27	0	—	—	3	—	—	—	—	—
150	3	3	0	24	0	—	—	3	—	—	—	—	—
200	3	3	0	21	0	—	—	3	—	—	—	—	—
300	3	3	0	18	0	—	—	3	—	—	—	—	—
450	6	6	0	15	0	100,0	0	6	—	—	—	—	—
500	6	2	4	9	4	69,2	30,8	6	—	—	—	—	—
550	3	2	1	7	5	58,3	41,7	3	—	—	—	—	—
600	4	3	1	5	6	45,4	54,6	4	0	9	0	100,0	0
700	5	2	3	2	9	18,2	81,8	4	1	5	1	83,3	16,7
750	4	0	4	0	13	0	100	1	3	1	4	20,0	80,0
800	6	0	6	0	23	—	—	0	6	0	10	0	100,0

best name for them is therefore "variation curves of resistance-tolerance" (or "resistance–tolerance curves" for short). The best name for curves B and B_1 is "rance–lethality curves".

Figure 3 shows that both the curves for the adult rabbits (A and B) slant more sharply to the right than the curves for the baby rabbits (A_1 and B_1). Both the A and B curves for the adult rabbits lie considerably to the right of the lethal dosage range for the physiologically mature baby rabbits. The size of the range of resistance for adult rabbits shown in Fig. 3(5-700 mg/kg) is notable, being considerably greater than the ranges of resistance and tolerance combined for baby rabbits. Conversely, the range of doses to which adult rabbits react by the second, reversible, reaction phase is not only narrower than their own range of resistance, but narrower than the range of tolerance for baby rabbits.

Their wider range of resistance indicates that the resistance of adult rabbits is much higher than that of baby rabbits, since maintenance of the original condition or increase in the lability of the thermoregulator, respiratory and cardiovascular systems and preservation of homeostasis can, under conditions of alteration, occur in the former within a wider range of effects than in baby rabbits.

One cannot conclude from the relatively narrow range of tolerance of the adult rabbits that, in spite of their higher resistance to chloral hydrate, their tolerance for it is less than that of baby rabbits. Such a paradoxical conclusion is impossible, due to the fact that the right margin of the range of tolerance (curve B in Fig. 3) is located within a higher range of doses than is the right margin of the range of tolerance for baby rabbits (curve B_1 on Fig. 3).

The data presented in this article allow one to regard the range of doses within which the maintenance of homeostasis is possible as a criterion of resistance, and the range of doses within which reversible disturbance of homeostasis is observed, as a criterion of tolerance.

A criterion based on the comparative values of the lethal doses is not adequate to compare sensitivity and resistance, nor can it fully characterize the differences in the tolerance of the very young organism for pharmacological substances.

Relying on the criteria mentioned, we established that in the case of chloral hydrate, both the resistance and tolerance of baby rabbits are lower than those of adult rabbits. The range of chloral hydrate doses within which the condition of homeostasis is maintained in adult rabbits is considerably wider than the range of resistance for baby rabbits. Moreover, the right margin of the range of resistance for adult rabbits lies between doses much higher than the absolute lethal doses for baby rabbits.

When comparing the sensitivity, resistance and tolerance of an organism at various ages in relation to any pharmacological substance, one must differentiate between these concepts and study the physiological reactions to doses ranging from the threshold to the lethal; research as to the effect of doses and concentrations lying within the range of resistance is highest in practical importance, as the latter approximates the concept of the therapeutic range.

SUMMARY

An attempt was made to differentiate the concepts and criteria of sensitivity, resistance and tolerance of animals of different ages using the example of chloral hydrate action. The concept of sensitivity is very close to the physiological notion of excitability; the minimal dose which provokes primary reaction on definite tissues or organs, to which other formations fail to react, should be regarded as a sensitivity criterion. The range of doses within which animals of various ages respond by reactions of increased functional mobility of the nerve centers and by maintenance of homeostasis is taken as a resistance criterion. Tolerance is to be understood as a condition of disturbed homeostasis, reversible under the action of a definite range of doses, this range being regarded as a criterion of tolerance. The criterion based on contrasting lethal doses is not adequate to draw a comparison between sensitivity and resistance in animals of various ages to characterize their tolerance.

LITERATURE CITED

1. I. A. Arshavskii, Uspekhi Sovremennoi Biol. 32, 18 (1951).
2. I. A. Arshavskii, Transactions of the Conference on Age Changes in the Metabolism and Reactivity of the Organism [in Russian] (Kiev, 1951) p. 158.
3. I. A. Arshavskii, Uspekhi Sovremennoi Biol. 34, 384 (1952).
4. V. M. Karasik, Byull. Éksp. Biol. i Med. 10, 206 (1940).
5. V. M. Karasik, Uspekhi Sovremennoi Biol. 17, 71 (1944).
6. I. A. Kornienko, Transactions of the Third Scientific Conference on Questions of Age Morphology, Physiology and Biochemistry [in Russian] (Moscow, 1959) p. 308.
7. K. A. Meshcherskaya-Shteinberg, Byull. Éksp. Biol. i Med. 7, 219 (1939).
8. K. A. Meshcherskaya-Shteinberg, Byull. Éksp. Biol. i Med. 7, 452 (1939).
9. K. A. Meshcherskaya-Shteinberg, Byull. Éksp. Biol. i Med. 10, 441 (1939).
10. K. A. Meshcherskaya-Shteinberg, Byull. Éksp. Biol. i Med. 11, 312 (1942).
11. V. D. Rozanova, Proceedings of the Seventh All-Union Convention of Physiologists, Biochemists, and Pharmacologists[in Russian] (Moscow, 1947) p. 699.
12. V. D. Rozanova, Fiziol. Zhur. SSSR 40, 453 (1954).
13. V. D. Rozanova, The Physiological Mechanisms of Particular Effects of Certain Pharmacological and Toxic Substances at Different Age Periods (Doctoral Dissertation) [in Russian] (Moscow, 1949).
14. V. D. Rozanova, Hypoxia [in Russian] (Kiev, 1949) p. 97.
15. V. D. Rozanova, Transactions of the Third Scientific Conference on Questions of Age Morphology, Physiology, and Biochemistry [in Russian] (Moscow, 1959) p. 314.
16. A. M. Rusanova, Farmakol. i Toksikol. 12, 39 (1949).
17. L. I. Tank, Farmakol. i Toksikol. 14, 48 (1951).
18. L. I. Tank, Byull. Éksp. Biol. i Med. 35, 38(1953).
19. L. I. Tank, The Organism's Tolerance of Pharmacological Substances at Different Stages of Postnatal Development (Author's Abstract of Doctoral Dissertation) [in Russian] (Leningrad, 1953).
20. N. B. Eddy, J. Pharmacol. J. a. Exper. Therap. 66, 182 (1939).
21. F. A. Falck, Arch. ges. Physiol. 34, 530 (1886).

EFFECT OF IONIZING RADIATION ON THE DEVELOPMENT OF IMMUNITY BY IMMUNIZATION WITH LIVE TULAREMIA VACCINE

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(Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Parin)

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In a previous communication we have already demonstrated that in conditions of severe radiation sickness an acute exarcerbation of the vaccinal tularemia infection occurs in mice and that this is associated with the residual virulence of the vaccine strain B. tularense [1]. In the case of guinea pigs a different picture was observed x-ray irradiation had no exacerbating effect on the vaccinal tularemia infection [2].

The present study is concerned with the action of ionizing radiation on the development of immunity by immunization with live tularemia vaccine.

EXPERIMENTAL METHOD

White mice with an average weight of 15-20 g were irradiated with a dose of 400 r. 504, 360, 192, and 24 hr before being subcutaneously injected with 50 bacterial cells of a 48-hr culture of the vaccine strain B. tularense isolated from dry, live tularemia vaccine (F. N. Gamaley Institute of Epidemiology and Microbiology of the Academy of Medical Sciences of the USSR, batch No. 459, control No. 8209), as well as 48 and 168 hr after infection with a similar dose of the vaccine strain. 480 hr after the injection, the animals were infected with a virulent strain B. tularense No.188 in a dose of 100 DCLM. At the same time the nonirradiated, vaccinated and unvaccinated control mice were also infected. The number of bacterial cells was determined by the intestinal optical standard.

Guinea pigs used weighed an average of 400-500 g. In the first test they were vaccinated subcutaneously with the vaccine strain and in the other 2 tests dermally with live tularemia vaccine (F. N. Gamaley Institute of Epidemiology and Microbiology of the Academy of

TABLE 1. Effect of Irradiation on the Course of Vaccinal Infection and the Development of Immunity in Guinea Pigs Vaccinated with Live Tularemia Vaccine (test No. 2)

Vaccination	Radiation dose (in r)	Number of guinea pigs	Type of vaccinal infection				Number of guinea pigs	Immunity tests	
			death rate of guinea pigs (after 720 hr)	average titre of agglutinins	vaccination: av infiltrate diam (mm) 192 hr after vaccination	vaccination: av duration of inflammatory reaction		dose of virulent strain of B. tularense (number of bacterial cells)	number of guinea pigs dying from tularemia
24 hr before irradiation	180	10	1	1:95	18,3	8,6	8	10 000	0
24 hr after irradiation	180	10	2	1:62	9,2	6,3	8	10 000	1
168 hr after irradiation	180	10	1	1:88	9,6	7,7	9	10 000	1
Control	—	10	0	1:144	13,1	9,2	10	10 000	0
"	—	—	—	—	—	—	3	1	3
"	—	—	—	—	—	—	3	10	3

TABLE 2. Effect of Irradiation on Vaccinal Infection and the Development of Immunity in Guinea Pigs Vaccinated with Live Tularemia Vaccine (test No. 3)

Vaccination	Radiation dose (in r)	Type of vaccinal infection								Immunity tests											
		number of guinea pigs	death rate of guinea pigs (after 720 hr)	av titre of agglutinins 600 hr after vaccination	allergic reaction (av diameter of the infiltrate in mm)	vaccination				number of guinea pigs	dose of the virulent strain B. tularense (number of bacterial cells)	number of guinea pigs dying from tularemia	killed after 720 hr						av titre of agglutinins
						av duration of the reaction (in hr)	av infiltrate diam (mm) at reaction height						due to festering of regional lymph nodes	from specific changes in internal organs	number of positive results			total	
															biological tests	sowings			
24 hr before irradiation . .	205	10	5	1:108	1,0	10	17,0			5	100	2	1	0	1	1		1	1:20
After irradiation :																			
24 hr after	205	10	2	1:52	2,6	6,8	13,4			8	100	2	6	0	1	1		6	1:113
144 hr after	205	10	4	1:113	1,3	6,0	10,7			6	100	0	6	0	0	0		6	1:120
296 hr after.	205	10	8	1:120	5,0	2,2	3,8			2	100	0	1	0	0	0		2	1:120
504 hr after	205	9	6	1:160	—	9,0	12,6			3	100	0	3	0	0	0		3	1:106
Control	—	8	0	1:80	4,0	9,4	19,0			8	100	0	5	1	4	4		8	1:75
"	—	—	—	—	—	—	—			14	1	14	—	—	—	—		—	—

Medical Sciences, USSR, batch No. 459, control No. 8209). Three scratches, each 2 cm long, were made in the depilated stomach surfaces of the guinea pigs and 3 drops of tularemia vaccine were rubbed in at different periods before and 24 hrs after irradiation. The radiation dose was in the range 180 r [LD-10-20(30)]to 224 r [DL-50-60 (30)]. The guinea pigs were then studied for the intensity and duration of the inflammatory reaction at the site of vaccination, the allergic reaction to the injection of tularin and the titre of specific agglutinins.

696-744 hr after vaccination the guinea pigs were subcutaneously infected in the right haunch with a virulent strain B. tularense No. 188. Those that died within 720 hr after testing for immunity were examined for pathological-anatomical lesions and sowings were made on a hard yolk medium, a bacterioscopy was carried out of the prints of the organs stained by the Romanovskii-Gimza method, and white mice were infected with the suspension of the organs. In the last test, all the surviving guinea pigs were killed by phlebotomy 720 hr after infection with the virulent strain, the titre of agglutinins in the serum was determined, sowings of the organs were made on a hard yolk medium, and white mice were infected with the suspension of the organs. Organs of the white mice that died were then sown on a hard yolk medium and used for the thermal precipitation reaction with antitularin serum.

The animals were irradiated in an x-ray therapeutic apparatus RUM-3. Conditions of irradiation were as follows : 180 kv, 10 ma filters 1 mm Al and 0,5 mm Cu, distance from the anode tube 40 cm, filed 20 · 20 cm, dosage 26-27 r/min.

Three tests were conducted on guinea pigs (using a total of 144 animals). In the first test(27 guinea pigs) the animals were subcutaneously injected with 10,000 bacterial cells of the vaccine strain over a period of 24 hr or 24 hr before irradiation with a large dose of x-rays (224 r) which caused death in 50-60% of the animals. In guinea pigs vaccinated 24 hr after irradiation a severe inhibition of the allergic reaction was observed and upon testing for immunity (infection 1000 DLM of virulent strain B. tularense) both the surviving guinea pigs of this group died from tularemia. Irradiation carried out 24 hr after vaccination did not have any definite effect on the development of immunity in this test.

Results of the second and third tests are given in Tables 1 and 2.

In these tests vaccinations were made dermally. As will be observed from the data in Tables 1 and 2, irradiation 24 hr before vaccination produced a slight inhibition of the immunological reactivity only in one of the 2 tests, whereas irradiation 336 and 504 hr before vaccination had no inhibiting effect on the intensity of immunity. Irradiation 24 hr after vaccination was

TABLE 3. Effect of Radiation on the Course of Vaccinal Tularemia Infection and on the Development of Immunity in White Mice

Mice	Before immunity tests						After immunity testing		
	number of mice	radiation dose (in r)	B. tularense vaccine strain dose (number of bacterial cells)	number of dead animals			no. mice infected with vir. strain B. tularense	B. tularense virulent strain dose (no. of bacterial cells)	no. of tularemia fatalities
				from tularemia	from radiation sickness and other causes	total			
Irradiation before the injection of the vaccine strain:									
504 hr before	20	400	50	4	10	14	6	100	0
360 hr before	20	400	50	1	8	9	11	100	4
192 hr before	30	400	50	8	8	16	14	100	1
24 hr before	30	400	50	11	14	25	5	100	1
Irradiation after the injection of the vaccine strain:									
48 hr after	20	400	50	8	5	13	7	100	2
168 hr after	20	400	50	2	7	9	11	100	6
Control	20	—	50	1	1	2	18	100	4
•	—	—	—	—	—	—	3	1	3
•	—	—	—	—	—	—	3	0,1	1

followed by inhibition of the development of immunity in one of the 2 tests (see Table 2).

Thus, dermal vaccination creates a very intense immunity and the process of its development is extremely radioresistant. The x-ray doses which produced an acute radiation sickness (with a decrease in the white cell count, 700-900 per mm^3), and caused death to a large number of animals, produced only a very slight inhibition of the development of immunity in infection with a massive dose of virulent tularemia microorganisms (1000-10,000 DCLM).

As will be seen from Tables 1 and 2 the degree of inhibition of the development of immunity in irradiated animals did not coincide with the degree of distinctness of the inflammatory reaction at the site of vaccination. Thus, irradiation 24 hr after vaccination did not weaken the inflammatory reaction at the site of vaccination but reduced the intensity of postvaccinal immunity.

From Tables 1 and 2 it will also be seen that in the presence of a radiation syndrome there is no definite parallelism between the intensity of the synthesis of specific antibodies as a result of vaccinal infection and the intensity of postvaccinal immunity except in guinea pigs vaccinated 24 hr after irradiation; in these, the antibody titre as compared with the control showed a certain fall and was accompanied by a decrease in the intensity of immunity. At the same time the inhibiting action of ionizing radiation on the development of postvaccinal immunity was associated with the inhibition of the allergic reorganization of the organism (see Table 2).

In all the experimental and control guinea pigs which had survived the immunity tests and were killed 720 hr after infection with the virulent strain B. tularense, no pathological lesions characteristic of tularemia in guinea pigs were observed in the internal organs. Nevertheless, in all the irradiated animals (with the exception of one guinea pig vaccinated 336 hr after irradiation) severe inflammation and purulent melting of regional lymphatic nodes characteristic of tularemia were found whereas among the vaccinated control guinea pigs these lesions were found only in 5 of the 8 animals.

Biological tests on mice showed that the organs of 4 of the 8 vaccinated control guinea pigs 720 hr after undergoing immunity tests, contained virulent tularemia bacteria; moreover, of 18 vaccinated guinea pigs which had been subjected to irradiation, virulent bacteria in the internal organs were found in only 2 animals (see Table 2). From these findings we may assume that inhibition of postvaccinal immunity provoked by the action of ionizing radiation is associated not with the suppression of the ability of the organism to render harmless virulent tularemia bacteria but with some other factors.

Table 3 embodies the results of the test carried out to study the action of x-ray irradiation on the development of immunity in white mice.

As will be observed from the data in Table 3, a radiation dose of 400 r which provoked death in 40-50% of the animals produced an acute exacerbation of the

vaccinal tularemia infection; this was particularly noticeable in mice irradiated 24 and 48 hr after the injection of the vaccine strain. On testing the immunity from a dose of virulent tularemia bacteria 100 DCLM the death rate from tularemia in mice irradiated at different periods before vaccination was no greater than in the vaccinated control animals which had not been irradiated.

Similar results were obtained for mice irradiated 48 hr after the injection of the vaccine strain. A distinct reduction in the immunity was observed only in animals irradiated 168 hr after injection of the vaccine strain.

Thus, our findings show that the process of the development of postvaccinal immunity by immunization with live tularemia vaccine has considerable radioresistance. These results likewise prove the absence of a definite parallelism between the ability of x-rays to provoke inhibition of immunity and their effect on the intensity of a number of immunobiological reactions (antibody synthesis, inflammatory reaction at site of vaccine injection). A more exact indicator of the degree of inhibition of immunity in irradiated animals is the allergic reaction to tularin injection.

SUMMARY

The process of formation of postvaccinal immunity against tularemia after immunization with the living tularemia vaccine is very radioresistant. Experiments were performed on guinea pigs. These animals were irradiated with various x-ray doses, ranging from 10-20 (30) LD to 50-60 (30) LD, at different periods before the vaccination and 24 hr after it. Such irradiation caused only a moderate reduction in intensity of the postvaccinal immunity. The process of postvaccinal immunity formation was found to be even more radioresistant in white mice, in which irradiation provoked an acute exacerbation of the vaccinal infection. The authors compare the ability of the irradiated and normal organisms to form immunity and the intensity of immunobiological reactions (antibody synthesis, inflammatory reaction at the site of vaccine injection, the ability of the tissues to render the virulent Past. tularensis harmless, allergic reaction to tularin).

LITERATURE CITED

1. A. S. Shevelev, Med. Radiol. No. 4, 50; A. S. Shevelev, Byull. Eksp. Biol. Med. 49, 60(1959).*

* Original Russian pagination. See C.B. translation.

ON THE EFFECT OF SUBCUTANEOUS ADMINISTRATION OF ANTITUMORAL SERUM ON THE DEVELOPMENT AND GROWTH OF THE TRANSPLANTED BROWN-PEARCE TUMOR

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Specific immune sera have been used in the last few years to determine the characteristics of the antigenic properties of tumors. With the help of these sera a number of authors [4, 5 and others] established that a single antigen specific for all cancerous tumors of the human does not exist. In the organs and tissues of a cancer patient that are not affected by tumor the "cancer antigen" is absent [6], and it is contained only in the tumor elements. By its chemical nature the tumor antigen presents itself as an intricate complex of amino acids [3]. The acquisition of monospecific sera in connection with the establishment of a "cancer antigen" [7, 8 and others] and their use for the treatment of a tumor process is, essentially speaking, only beginning [2, 9, 10], and would seem to open new possibilities in the treatment of cancer. This was well expressed by V. S. Gostev [1] in the following words: "The acquisition of monospecific antisera and monospecific antibodies will set the stage for a successful solution to the problem of cytotoxinotherapy, in particular to the problem of the action of antibodies on the malignant growth of tissues in the cancer afflictions." In our investigations we did not begin from the idea of obtaining sera which would contain only antibodies specific for tumor antigen. The prevalent idea of the necessity, as much as possible, of enriching the obtained sera with specific antibodies and getting rid of "ballast" substances scarcely reflects the complex immunological mechanisms determing the resistant properties of the organism to the disease in general, and, in particular, to the tumor process. The fact is that the biological characteristics of tumors are related, in a close manner, not only to the species specificities, and reflect not only these, but also to the properties obtaining in animals of other species. Thus, in the tumor process to a greater or lesser degree there appear mechanisms departing from their roots deep in the evolutionary development and establishment of the organism. These peculiarities also determine the unique reactive properties of the organism as a whole, and are expressed in the formation of characteristics of the individual tumor process. The viewpoint of V. S. Gostev noted above, reflecting the contemporary direction in cytotoxinotherapy, is one-sided, and the expected therapeutic effect from such sera is related to their selective action on the tumor process.

Preparing the antitumoral sera, we first took into account the role of the nervous system in the phenomena of immunity, and especially its receptor link, inasmuch as this dictated the necessity of including in the immunobiological reactions the basic barriers of the organism from the aspect of its evolutionary development and establishment. Secondly, we directed our attention to the structure and function, comparable to a mosaic, of tumor tissue (the presence of different antigens in the same tumor) and the possibility of differentiating the tumor elements. All these gave us a basis for speaking of the necessity of all the "specific" and "nonspecific" antibodies being present in the obtained sera, having a wider range of activity in the fundamental mechanisms giving rise to, and elevating, the resistant properties of the organism.

EXPERIMENTAL METHOD

We obtained sera from the blood of dogs which were immunized with a suspension of the Brown-Pearce tumor in physiological saline. The sera were assayed in biological tests, whereby the action of the sera in these tests was accompanied by inhibition of cell division and symptoms of hyperplasia of organs rich in reticuloendothelium. Investigations were carried out on the transplantable strain of Brown-Pearce rabbit tumor. Fifty-six male rabbits were used for the experiments (35 experimental and 21 control). The transplantation was performed in the left gonad by means of introducing 0.5 ml of a tumor suspension in physiological saline. After 18 days from the moment of this inoculation we began to inject the experimental group 2–3 times per week with the antitumoral serum on the basis of 0.3 ml per kilogram of body weight. In the rabbits at that time it was possible to see an increase in the size and density

of the afflicted gland, and a random mortality of the animals (Table 1) in the first 2–3 weeks passing after the moment of inoculation testified to the magnitude of injury of the internal organs. In Tables 1 and 2 there is included only a part of the material, since changes of analogous presentation were observed in the remaining animals.

EXPERIMENTAL RESULTS

In the control group (see Table 1) we observed no cases of spontaneous resorption of the tumors. The animals in this group lost weight, the process clearly progressed (a marked increase was noted in the size of the sex gland, it was possible to feel rod-like extensions emanating from appendages in the pelvic region, clusters of nodes in the peritoneal cavity, etc.), and at autopsy this was confirmed on occasion by marked metastatic involvement of almost all the internal organs. In the experimental group we attempted to treat the tumor-bearing animals to the point of clinical recovery (normalization of the blood picture, increase in weight, absence of metastases in a randomly sacrificed animal, etc.).

Using microscopic investigation it was discovered that the tumor consisted of epithelial elements, circular in form, lying very close together and not possessing a natural stroma. Not infrequently there were encountered foci of hemorrhages and here and there, vessels of the capillary type. The tumor clusters tended toward a central necrosis. In the area of necrotic foci there was observed karyorrhexis, pyknosis, and occasionally vacuolization of the cell nucleus. The tumor cells grew destructively and in an infiltrating fashion. Along with the progression of the tumor process a leukocytosis developed (attaining up to 18,000 to 20,000 at the end of the animal's life) involving the neutrophilic forms and with a concomitant decrease in the lymphoid elements. In the course of the rabbits' recovery their weight usually increased, and only in those cases where the process continued, although involvement was limited to only the surrounding tissues, did the weight not only not increase, but significantly fall (Table 2).

Along with the administration of the serum, the duration of the animals' life increased, and on autopsy it was possible to note a complete absence of visible metastases and a resorption of the original focus. However, the fact is noteworthy that in those cases where the original focus existed for a prolonged period of time, metastases in the lungs, diaphragm, and kidneys were persistently retained as well. Apparently, it makes sense at a given stage of treatment to remove the primary focus and secure the liquidation of metastases as fast as possible. As the amount of administered serum was increased at times, the involvement of the organs progressively decreased, especially those which are most quickly involved by metastases (see Table 2). In the process of resorption of the metastases it was possible to observe how the metastases, in the form of succulent whitish knots jutting out on the surface of the organ, then acquired a faded white, and subsequently yellowish, color. At that point they did not protrude on the surface of the organ or tissue and they were slightly deformed.

Upon microscopic investigation during the process of resorption both the primary focus and the metastases drew attention to a phenomenon which was described by us earlier in our study of the role of the nervous system in the tumor process. A reaction of the mesenchymal elements, the microphages (macrophages) was absent, growth of the connective tissue elements was noted, etc., and in the meantime, amidst the mass of tumor cells, fields of lysed tumor elements were encountered, in which were disseminated remnants of nuclear substance, separate preserved nuclei or their shadows. Here and there parts were found, presenting themselves as conglomerates of swollen tumor cells with coarse clumps of nuclear substance, staining intensely with hematoxylin, creating the impression of a peculiar "condensation" of nuclear material inside the cell protoplasm.

We postulate that in the internal milieu of the organism there appear oncolytic factors which are not only capable of causing lysis of the tumor cells but also of the unique process of "condensation" of the nuclear material; as a consequence of this the vital properties of the tumor are markedly disrupted and the action of enzymatic origin is facilitated, guaranteeing the further molecular disintegration of the living material in the tumor cells and their resorption.

The resultant reactive shifts in the organism appear in the blood as well. If, before the administration of the serum, there existed a tendency toward the upgrowth of a leukocytosis involving the neutrophilic forms and a considerable decrease in the lymphoid forms, then with regular administration of the serum the leukocytosis subsided and the relative composition of neutrophilic forms gradually fell; in conjunction with this the lymphocytic series rose, and even isolated lymphohistiocytic cells appeared. Thus, the blood picture gradually returned to normal.

SUMMARY

Brown-Pearce tumor was inoculated to experimental rabbits. Serum obtained from dogs immunized with suspension of Brown-Pearce tumor in physiological saline was injected to 35 experimental rabbits (2–3 times a week, 0.3 ml per kg of body weight). No serum was administered to 21 control animals inoculated with Brown-Pearce tumor. Individual rabbits of experimental groups were sacrificed during the process of treatment; the surviving experimental animals recovered in time as a result of serum treatment. Individual control animals were sacrificed too, whereas the rest of the rabbits of this

TABLE 1. Experimental Data (control group)*

Color of the rabbit	Weight of the rabbit (g)		Duration of life (in days)	Sites of metastatic involvement															Comments
	before inoculation	at time of death		hearts	lungs	diaphragm	epiploon	gastric serosa	spleen	liver	small intest. serosa	lge. intest. serosa	mesentery	adrenal	kidney	peritoneum	urinary bladder	gonad	
Gray	1 770	1 620	17	—	+++	—	++++	—	—	++	—	+	+	—	+++	+	—	++++	—
»	2 300	2 200	19	—	+	++++	++++	—	+	++++	++	++	++	—	+ ++	++	—	++++	—
»	2 300	1 700	27	—	+	+	++++	—	—	+	—	+	+	—	++	+	—	++++	—
White	1 910	1 780	17	—	+	++	++++	+	—	+	+	+	—	—	—	+	—	++++	—
»	2 300	2 110	30	—	+	+++	++++	+	—	+++	+	+	++	—	—	++	—	++++	—
»	1 710	1 600	25	—	++++	+++	++++	—	—	+	+	+	+++	—	+	—	—	++++	—
Gray	1 930	1 580	23	—	++++	+++	++++	—	—	++	—	—	++	—	+++	++	—	++++	—
Black	2 730	2 630	18	—	—	+++	++++	—	—	+	+	+	+++	—	—	++++	—	++++	—
White	1 920	1 820	16	—	+	+++	++++	—	—	+	++	++	+++	—	—	++	—	++++	Sacrificed (16 days)
»	1 950	1 930	30	—	—	+	++++	—	—	—	++	++	+++	—	+	++	++++	++++	—
»	2 700	2 460	45	—	+	++	++++	—	—	++	+	+	+	—	+++	++	—	++	—
Gray	2 400	1 950	20	—	—	+	++++	—	—	+	++	++	+	—	—	+	—	++++	Sacrificed after 20 days
White	2 150	1 900	12	—	—	++++	++++	—	—	++	++	++	++	—	++	++	+	++++	Sacrificed after 12 days

* Arbitrarily, for the expression of the degree of involvement of the organs or tissues by tumor, we adopted the following symbols: Isolated focus +, a group of foci ++, involvement of 3/4 +++, involvement of the entire organ ++++, and lack of involvement −(minus).

TABLE 2. Experimental Data (experimental group)

Color of the rabbit	Weight of the rabbit (in g)		Initiation of treatment (in the numerator), number of administrations of antitumoral serum (in the denominator)	Sites of metastatic involvement															Duration of life (in days) at the time of sacrifice
	before inoculation	at time of death		heart	lungs	diaphragm	epiploon	gastric serosa	spleen	liver	small intest. serosa	lge. intest. serosa	mesentery	adrenal	kidney	peritoneum	urinary bladder	gonad	
White	2 650	1 850	18th day / 4	—	+	++++	—	—	—	—	—	—	+++	—	+	++	—	++++	27
Gray	1 900	2 050	18th day / 5	—	+	+++	++++	—	—	++	+	+	+	—	++		—	++++	34
	2 150	2 470	18th day/18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	195
White	2 300	2 530	18th day / 13	—	—	—	+	—	—	—	—	+	+	—	++	+	—	++	62
"	2 400	2 410	18th day/ 16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	77
"	2 330	1 900	18th day/3	—	+	+	++++	—	+	—	—	—	+	—	+	+	—	++++	27
"	1 830	2 130	18th day/7	—	—	—	—	—	—	—	—	—	—	++	—	—	—	++	175
"	1 600	1 730	18th day/15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	91
"	2 300	2 000	18th day/7	—	+	+	++	—	—	—	—	—	+	—	++++	—	—	++++	39
"	2 050	1 900	18th day/13	—	+	—	—	—	—	—	—	—	—	—	+	—	—	++	69
"	1 830	2 050	18th day/17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	196
"	2 030	1 620	18th day/15	—	—	—	—	—	—	—	—	—	—	—	++++	—	—	++	101
"	1 900	1 330	21st day/7	—	—	+	++++	—	—	—	—	—	+	—	++	+	—	++++	41
Gray	1 700	1 580	21st day/2	—	+	+++	++++	—	—	—	—	+	++	—	+	+	—	++++	28

group perished 17 to 45 days after the inoculation of the tumor. Resorption of the primary focus and metastases was manifested macroscopically in the aquisition of faded yellow color by the tumor foci, their flattening and tendency to become deformed. Microscopically the primary foci and metastases exhibited the presence of the fields of tumor cell lysis, alternating with the areas of tumor cells in conditions of swelling and "condensation" of the nuclear substance, exteriorly to the cellular protoplasm.

LITERATURE CITED

1. V. S. Gostev, Vestn. AMN SSSR No. 4, 39 (1956).
2. V. N. Dobrokhotov, Byull. Éksp. Biol. i Med. 39, 59 (1955).
3. V. S. Korosteleva, M. I. Karlina, and N. V. Cheburkina, Byull. Éksp. Biol. i Med. 42, 44 (1956).*
4. V. S. Korosteleva and P. N. Kosyakov, Byull. Éksp. Biol. i Med. 43, 83 (1957).*
5. P. N. Kosyakov, V. S. Korosteleva, and N. I. Kuznetsova, Byull. Éksp. Biol. i Med. 40, 63 (1955).
6. P. N. Kosyakov and N. I. Kuznetsova, Byull. Éksp. Biol. i Med. 43, 49 (1957).*
7. A. K. Saakov, Byull. Éksp. Biol. i. Med. 34, 61 (1952).
8. A. K. Saakov, Byull. Éksp. Biol. i Med. 38, 53 (1954).
9. P. P. Filatov, Byull. Éksp. Biol. i Med. 40, 56 (1955).
10. P. I. Tseitlin and M. M. Kapichnikov, Byull. Éksp. Biol. i Med. 40, 60 (1955).
11. V. A. Chepurin, Trudy Tomskogo Med. Inst. 14, 51 (1945).

* Original Russian pagination. See C.B. translation.

THE EFFECT OF OVERSTRAINING THE SENSORY EXCITATION PROCESS ON THE REGENERATION OF MUSCLE TISSUE IN WHITE RATS

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The task of this work was to clarify the effect of a pathological condition in the process of cortical-stimulation within the cerebral hemispheres of the brain on the regeneration process in skeletal muscle. This question has still not been illuminated in the literature.

In experimental animals, under conditions overtaxing the strength and adaptability of the cortical excitation process, we studied the basic phases of regeneration in muscle tissue following partial transection of the muscle: The course of the inflammatory reaction, the destructive and proliferative phenomena, and the dynamics of growth and differentiation in the connective and muscle tissues.

EXPERIMENTAL METHOD

The experiments were carried out on 42 white rats, 300 to 400 g in weight, of which 20 served as controls and 22 as experimental animals. Before the operation the experimental rats were prepared according to the motor-alimentary conditioned reflex method (the task was carried out with each rat individually). The conditioned reflexes were worked out in special chambers with a moveable floor. The magnitude of the conditioned reflex was gauged by two indicators: the rapidity of the run to the food and the force with which the rat depressed the disk with the food, tripping the pneumatic system. A kymograph record reflected the following experimental data: the free motion of the rats in the chamber, the conditioned signals, the moment that the food was taken and the time in seconds.

Initially, positive motor-alimentary conditioned reflexes were worked out in the rats in response to an electric tone of average intensity and to a light from a 10 w electric lamp. After fortification of the conditioned reflexes we used an electric tone of greater intensity and a 150 w electric lamp. The number of combinations was increased, in one experiment, to 15 to 20, and the pauses between the conditioning signals shortened to 20 to 180 sec. Sterotype was excluded both in the order of the afforded stimuli and in their number or the pauses between them. From the conditions of the experiment it is apparent that the strength of the process of excitation in the rats was overtaxed, as was its mobility in both analyzers, visual and auditory. The indicated activities were applied for 20 days prior to the operation on the muscle and continued after the operation up to the time the material was taken for histological investigation.

Trauma was inflicted on the anterior tibial muscle, not including the basic nerves and vascular trunks. The muscle was cut with scissors through 2/3 of its thickness The operation was performed under strict aseptic conditions using ether anaesthesia. Material was fixed in Zenker formol, 12, 24, and 48 hr, and 5, 8, 12, 16, 20, and 30 days, after the operation. Serial paraffin sections were stained with appropriate histologic stains.

EXPERIMENTAL RESULTS

Upon working out the conditioned reflexes, differences were detected in the type-specific properties of the rat nervous system. Two basic groups appeared: the first with a predominance of the excitatory process, the excitable type; the second with predominantly inhibitory processes, the inhibited type. Both groups included variations which we did not separate out here.

With prolonged application of only intense positive stimuli in the majority of the rats in the first group the excitatory process acquired a pathologically inert character that, apparently, set the conditions for a strong excitation of the motor analyzer and a deficiency in the inhibitory process. In rats of the second group there was observed a temporary disappearance of the conditioned reflexes in the presence of a constant fortification of the conditioning signal, an incompletion in the conditioned reflex act, and a stiff posture, in which the rat remained over the course of several minutes, not reacting to the conditioning signals. In the given case, obviously, there occurred an intense inhibition of the motor component in the alimentary reflex. Phase phenomena were noted in the rats of both groups. Disruption of the vegetative functions was manifested by urination and defecation during the experiment. Several of the rats showed remarkable work capacities, manifesting them in prolonged maintenance of a high level of effort, even when we applied up to 20 combinations in one experiment.

In the rats of the control series 12 hr after injury the muscle fibers markedly swelled, lost their specific structure, and fell into fragments of different sizes.

An intense accumulation of leukocytes occurred in the wound exudate and between the injured muscle fibers; the predominant forms at this time were neutrophils. After 24 hr an active process of differentiation of cell forms from hematogenous and tissue origin into macrophages was noted, and along with this many neutrophils degenerated. After 48 hr the dead cells and necrotized tissue were almost completely resorbed; the fibrin was partially resorbed. In the central portion of the wound (the site of direct injury) the appearance of a thin latticework was maintained for 6 days. The wound filled in with granulation tissue; in this tissue the predominant forms were fibroblasts.

By means of complex reorganization, accompanied by dedifferentiation of the muscle fibers, accumulation of basophilic sarcoplasm, and multiplication of nuclei, the ends of the injured muscle fibers were converted into muscle buds and myosimplasts. They appeared to be the basic origins of muscle tissue reconstruction. On the fifth day there was observed an active formation of muscle buds and myosimplasts, growing into the granulation tissue in the form of long basophilic projections, tapered or swollen at the free end. The process of differentiation of the myosimplasts in the muscle tubes occurred most intensively on the 6th to 8th days. On the 12th day the undifferentiated myosimplasts remained very few in number, and the granulation tissue was replaced by loose connective tissue. Further development of the regenerative process was accompanied by the formation of collagen bundles, and by the growth and final differentiation of the young muscle fibers. After 20 days the process of collagen formation diminished at the same time that a marked increase in growth was noted in the regenerating muscle tissue. On the 30th day the defect was filled in with young muscle fibers, scattered in a disoriented fashion in the central portion of the wound, and small layers of connective tissue. The amount of connective tissue progressively decreased with the further formation of the regenerate.

Corresponding with the observations of Z. P. Ignat'eva [5] on the results of the reconstruction of skeletal muscle in rats followed for some time, after more than 2 months the site of the defect is just barely recognizable by the noticeable diversion in the path of the muscle fibers.

In the experimental rats of the excitable type with overstraining of the cortical process of excitation the extent of the destructively altered portions of injured muscle fibers was significantly greater than in the control rats. The inflammatory reaction was accompanied by copious exudation and more abundant transudation of leukocytes from the blood vessels. The increased exudation set up conditions inhibiting the phagocytic activity of the macrophages. Despite the vast numbers of the latter, after 2-3 days there was still much unresorbed detritus in the wound. In the maturing granulation tissue there was a multitude of polyblasts and newly formed capillaries, the calibre of which was significantly increased by the 5th day (Fig. 1 a, b). The regenerative processes in the connective tissue at that period were manifested more more weakly than in the control rats. The fibroblasts had the form of small cells with poorly developed extensions, loosely disposed. An increased proliferation of nuclei was noted in the muscle buds and myosinplasts. On the 8th day a shift occurred in the regeneration reaction of the connective tissue toward an increase in collagen formation, as a result of which the defect quickly filled in with loose connective tissue. On the 12th day a considerable amount of differentiated connective tissue was formed, which had a negative effect on the growth of the newly formed muscle. There was especially large amounts of connective tissue in the central portion of the wound, where the young muscle fibers underwent complete atrophy. In addition, local growth of the intermuscular connective tissue and fascia took place.

These formations were made up of collagen fibrils, cellular elements, blood vessels, and thin (4 μ in diameter) but completely differentiated muscle fibers.

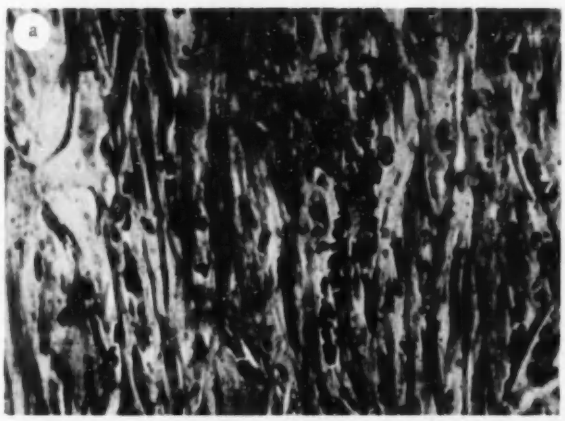
On the 16th day the composition of the scar components pointed to a profound trophic disturbance in the regenerating muscle tissue. Chaotically growing massive collagen bundles compressed the atrophically altered young muscle fibers. Granular clumps were visible—remains of the destruction of the latter, surrounded by lymphoid elements (Fig. 2a, b). On the 30th day the muscle wound was filled in with well-developed collagen bundles, forming complex interweavings with the poorly developed young muscle fibers. The latter varied in diameter (from 4 to 20 μ) and in the state of their differentiation, while the fibers with the smallest diameter had the most clearly manifested transverse striations.

Thus, in the excitable rats, the regeneration of muscle tissue is characterized by the following fundamental features: intense development of the inflammatory process, increased regenerative reaction on the part of the blood vessel system, increased collagen formation after the 8th day of healing and inhibition of growth of the young muscle fibers.

In regard to the experimental rats of the inhibited type, overstraining of the process of excitation reflected itself on the regenerative process in a different way. The inflammatory reaction in the work-capable rats of the inhibited type ran its course in a manner similar to the controls, which, apparently, was related to the development of a protective inhibition in them. In rats of the extremely inhibited type, in which the process of excitation was also weak, the inflammatory reaction distinguished itself in its sluggishness and its lingering character. Twelve

hours after the operation there were very few leukocytes in the wound; at the end of the first day the inflammatory picture had almost not changed, inhibition being noted in the differentiation of the cellular elements into macrophages. On the 8th day the edema was still not resorbed, the fibroblasts were poorly differentiated, and many underwent degenerative changes. The myosimplasts were vacuolated, and stained poorly as a result of cloudy swelling. These disturbances appeared to be a result of the extreme debilitation of cortical activity, caused by overstraining of the excitatory process.

In the work-capable rats of the inhibited type the regenerative reaction of the muscle tissue showed itself as an intense accumulation of sarcoplasm at the ends of the old injured muscle fibers. This gave rise to massive beadings, yielding a weakly basophilic reaction. The differentiated structures appeared in them on the 8th to 12th days, in the form of loosely scattered myofibrils. Depression of the neurotonic state by means of systematic overstraining of the excitatory process led to inhibition of the differentiation in the regenerating tissue. On the 16th day portions of unreplaced granulation tissue remained



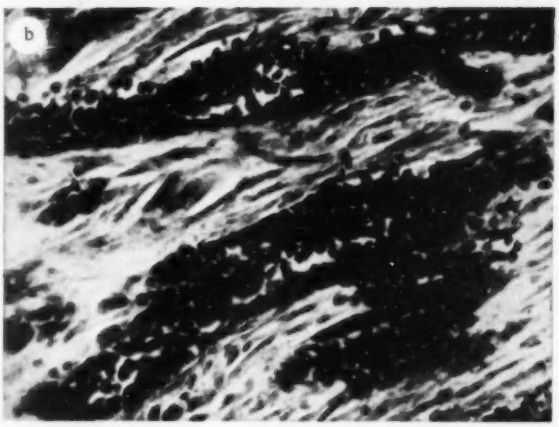


Fig. 1. Photograph of the healing muscle injury on the fifth day after operation. a) In the control rat. The wound is filled in with granulation tissue and the myosimplasts growing in it. The newly formed blood vessels are visible; b) in the rats of the excitable type, with overstraining of the excitatory process. The granulation tissue is intensively vascularized, and the c calibre of the newly formed blood vessels is greatly increased. Stained with azure-II-eosin. Magnification: Objective 40 ×, ocular 7 ×.

Fig. 2. Healing of the muscle wound on the sixteenth day after operation. a) In the control rat. The wound is filled in with regenerated muscle fibers and sheets of loose connective tissue; b) in the rats of the excitable type with overstraining of the excitatory process. Coarse fibered buds of connective tissue are visible in the wound, as well as atrophic changes in the yound muscle fibers and granular clumps, traces of their destruction. Stained according to Mallory. Magnification: objective 40 ×, ocular 7 ×.

in the wound; on the 20th day there were many young muscle fibers that did not have cross striations, although they surpassed the old fibers in diameter (26 μ against 22), and, in addition, a vigorous multiplication of nuclei occurred in them. Myosimplast formation was encountered among the young muscle fibers, as well as many vessels and large groups of lymphoid cells.

On the 30th day the wound defect was filled in with young muscle fibers, 24 to 30 μ in diameter (the old fibers had a diameter of 22-24 μ), which contained many nuclei, oval in form. The connective tissue was more poorly developed than in the control rats. Far from the wound it was possible to see fine regeneration foci, arising, obviously, as a result of secondary necrosis.

Our data shows that the regenerative process in the muscle of white rats subjected to overstraining of the cortical process of excitation runs its course in a varying fashion, depending on its type-specific characteristics. Thus, in lower mammals the inherent functional structure of the cortical cells holds a significance in the reaction of the organism to external influences.

The basis of the changes in the picture of injured muscle regeneration in the experimental rats lies in the trophic disturbances caused by overstraining of the excitatory process.

According to the learned K. M. Bykov, disturbances originating in the neurotonic state protect not only the cells of the cerebral cortex but also the subcortical centers, and thus influence the metabolism, alter the tissue properties, the tissue properties, the permeability of blood vessel walls , etc.

This hypothesis has been supported numerous times in the literature [2, 10, 12]. A close relationship has been established between the functional state of the cerebral cortex and the separate components of the inflammatory reaction: exudation, transudation of leukocytes, alterations in the permeability of blood vessels [3, 4, 7, 8, 11].

Our data shows that disruption of the normal cortical activity in animals influences the course of the inflammatory reaction and the entire intricate complex process of traumatic regeneration in skeletal muscle.

SUMMARY

The author investigated the regeneration of the anterior tibial muscle in white rats the nervous system of which was subjected to stimulation.

In overstraining the cortical process of excitation in white rats with an excitable type of the nervous system the inflammatory reaction of the anterior tibial muscle is accompanied by extensive disintegration of the injured tissues, increased exudation and infiltration of the area of injurity with leukocytes. The granulation tissue shows marked vascularization. Restoration of the muscular tissue is inhibited by the active connective tissue development. The regenerative reaction in rats with a weak excitation process,i.e., in the animals with extreme inhibition, is depressed. In rats with a stronger nervous system (inhibitable, but capable of functioning) the inflammatory reaction follows the course similar to that in control animals. Intensification of the neurotic condition induces a delayed differentiation process in the connective and muscular tissues.

LITERATURE CITED

1. K. M. Bykov, The Cerebral Cortex and Internal Organs [in Russian] (Moscow-Leningrad, 1947).
2. K. M. Bykov, Zhur. Vyssh. Nervnoi Deyat. 5, 449-462 (1955).
3. A. A. Vishnevskii, Khirurgiya, No. 7, 37-46 (1953).
4. E. Ya. Zakhariya, The Effect of Intense Stimulation and Maximum Inhibition on the Initiation and Course of Inflammation (author's abstract of Dissertation) [in Russian] (Lvov, 1953).
5. Z. P. Ignat'eva, Doklady Akad. Nauk SSSR 66, 285-288 (1949).
6. A. I. Karaev, T. D. Gaibov, and S. Ragimova, Doklady Akad. Nauk. Azerbajan SSR 11, 433-436 (1955).
7. A. I. Karamyan, Fiziol. Zhur. SSSR No. 5, 561-579 (1953).
8. N. E. Kovaleva, Doklady Akad. Nauk SSSR 103, 925-928 (1955).
9. I. P. Pavlov, Complete Works 3 [in Russian] (Moscow-Leningrad, 1951) book 2, pp. 37-48.
10. M. K. Petrova, On the Role of a Functionally Weakened Cerebral Cortex in the Genesis of Various Pathological Processes in the Organism [in Russian] (Moscow, 1955).
11. A. F. Sukhanov, The Effect of Partial Removal and Chronic Injury of the Cerebral Cortex of White Rats on the Processes of Proliferation in Foci of Inflammation (Author's Abstract of dissertation) [in Russian] (Moscow, 1954).
12. M. A. Usievich, Studies of I. P. Pavlov in Theoretical and Practical Medicine [in Russian] (Moscow, 1951) pp. 92-105.

REPARATIVE REGENERATION OF SKELETAL MUSCLE IN MAMMALS RECEIVING VITAMIN B_{12}

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With the increasing clinical use of vitamin B_{12}, its application is no longer confined to disorders of hemopoiesis, but it has been extended to the treatment of of many other diseases, and, in particular, of neuromuscular dystrophies [12] and diseases of the peripheral nervous system [6, 9]. Several workers have reported [1, 2, 4, 7, 8] that vitamin B_{12} accelerates the regeneration of peripheral nerves after trauma, which is bound to have a benefical effect on reparative processes in damaged muscle. As Charkey and his co-workers [11] have shown, the administration of vitamin B_{12} to an animal causes an increase in the synthesis of tissue proteins, especially in skeletal muscle.

In view of these findings, we considered it desirable to study the effect of vitamin B_{12} on the regeneration of skeletal muscle tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male white rats, weighing from 200 to 250 g. Vitamin B_{12} was injected intramuscularly in a dose of 20 μg to each animal. For the first 20 days the injections were given daily, and thereafter on alternate days.

In selecting this dose we were guided by the findings of Leroy and Robin [12], who showed that high doses of vitamin B_{12} were therapeutically effective in neuromuscular disorders. In addition to vitamin B_{12}, one group of experimental animals was given thyroidin by mouth. A group of animals acted as controls.

The object selected for study was the tibialis anterior muscle in both hindlimbs. Operations were performed under ether anesthesia in strictly aseptic conditions. After exposure of the muscle, a small hole was excised in its center with a special round punch (5 mm in diameter) at an equal distance from the point of attachment of the tibialis anterior muscle to the crest of the tibia. To prevent injury to the underlying tissues, a thin metal plate was introduced beneath the muscle. Sutures were inserted into the skin at the wound edges. The muscles were fixed in Zenker formol at different times during the month. Sections in paraffin wax were cut to a thickness of 6-8 μ, and stained with Carazzi's hematoxylin, azure II-eosin, Mallory's mixture and iron hematoxylin.

EXPERIMENTAL RESULTS

The distinguishing feature of the reparative manifestations in the control rats was the slight spread of the destructive processes in the injured muscle. In the proximal portion of the wound, for instance, only the ends of the divided muscle fibers underwent degenerative changes; in the distal portion the muscle fibers immediately injured were in process of destruction.

On the fourth day after operation the products of destruction had completely resorbed. The defect in the muscle was filled with an exudate containing fibrin, effused blood and numerous cells of tissue and vasogenic origin; granulation tissue was developing along the periphery of the wound. At this stage, signs of regeneration of the muscle tissue were already well marked. In the proximal portion of the wound, muscle buds were growing out from the viable ends of the muscle fibers, and becoming outstretched into myosymplasts. The absence of conditions favoring stretching in this area, however, led to a haphazard arrangement of the developing buds and myosymplasts. Their volume increased as a result of the growth in the mass of the cytoplasm, but their increase in length was slowed down. At the distal end of the wound, the newly formed muscle cells mainly had the form of thin, stretched myosymplasts, arranged along lines of tension arising in this particular area of the muscle, functioning more actively. Myofibrils appeared in the sarcoplasm of the myosymplasts; differentiation into Q and I disks could be observed in some of them.

Seven days after operation the center of the defect still contained much exudate, with fibrin and blood cells. At the periphery there was intensive proliferation of granulation tissue, undergoing differentiation into loose connective tissue. At this stage the myosymplasts continued to develop into muscle tubes. On the tenth day the nuclei in the muscle tubes began to be displaced beneath the sarcolemma, which was due to the process

of formation of the muscle fibers. At the end of the wound nearest the tendon, more fibers were formed. The number of cells in the connective tissue decreased, and dense collagen bundles appeared.

Fifteen days after operation, adipose tissue could be seen in the center of the defect, replacing the round-cell infiltration. Differentiation of the muscle tubes into young muscle fibers continued. On the twentieth day the processes of their formation were largely terminated; the newly formed muscle fibers were distinguishable from the old by the larger number of nuclei and their smaller diameter. From 25 to 30 days after operation, the defect in the muscle was filled with a chaotic disarray of muscle fibers (Fig. 1). Much of the regenerated tissue consisted of connective tissue, which had acquired the character of compact fibrous tissue.

At the stage of 4 days, in the experimental animals receiving vitamin B_{12}, more cells had accumulated in the region of the wound. Granulation tissue filled nearly the whole of the defect, and only in the center of the cavity were exudate and fibrin still present.

The reparative processes in the muscle tissue of the experimental animals were characterized by sharply defined reactive changes in those muscle fibers not directly damaged. These changes took the form of increased destruction of part of the muscle fibers, and especially of the intensive morpho-physiological reorganization of other muscle fibers, with the active formation of young cells. In spite of the widespread nature of the destructive changes, on the fourth day after operation the zone of injury was completely free from products of destruction, which was evidence of the high intensity of the processes of resorption. At the site of the resorbed necrotic masses, many myosymplasts had arisen from the remaining viable fragments of muscle fibers. In the experimental animals, in contrast to the controls, the processes of dedifferentiation (the formation of sarcoplasm rich in nucleic acids) and the regenerative increase in the number of nuclei extended to a considerable number of muscle fibers, especially in the lateral and distal portions of the muscle. Such "excited" fibers became the source of formation of an abundance of myosymplasts (Fig. 2). The latter arose as the result of longitudinal splitting of muscle fibers with the isolation of long bands of basophilic cytoplasm from the nuclei, as we have previously described in hyperthyroid animals [5]. This method of formation of the myosymplasts brings about their parallel arrangement, which also provides for preservation of the endomysium.

In the experimental rats at this stage, just as in the control animals, differentiation of the sarcoplasm began in the myosymplasts. The development of muscle cells was most intensive in the areas where they were formed by cleavage from the "excited" muscle fibers of long myosymplastic bands. Mitosis was more often found in the myosymplasts in rats receiving vitamin B_{12} than in the control animals.

Subsequently, on the 7th-10th day, some of the myosymplasts had become converted into muscle tubes which, in turn, developed into young muscle fibers. On histological examination of the regenerating tissue 10 days after injury, attention was drawn to the active growth of the muscle tubes and fibers. The distal and lateral

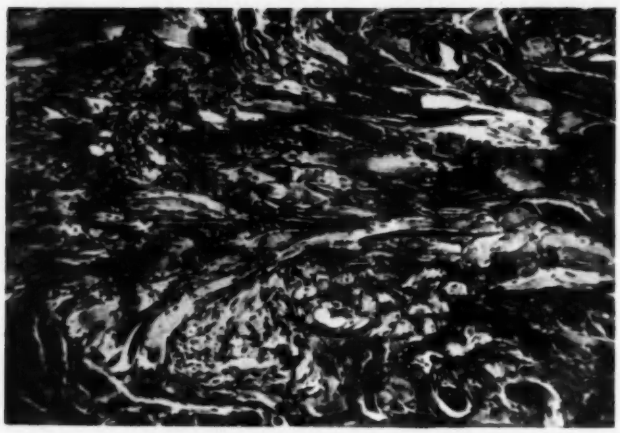

Fig. 1. Defect in the muscle 25 days after operation. Control. Zone of repair nearer the proximal end of the wound. The defect in the muscle is filled with a chaotic disarray of muscle fibers. Stained by Carazzi's hematoxylin. Magnification: ocular 10×, objective 8× .

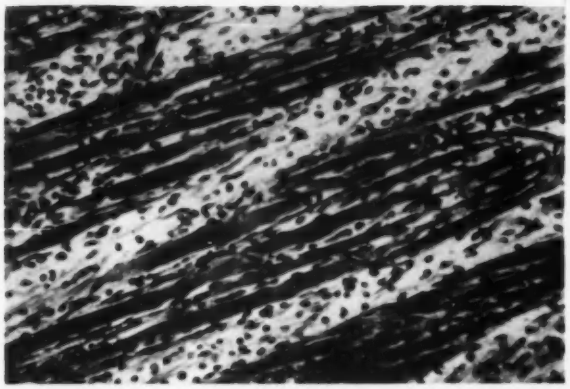

Fig. 2. Defect in the muscle 4 days after operation. Experiment using vitamin B_{12}. Growth of myosymplasts in areas of reactive reorganization of muscle fibers at a distance from the wound. Stained by Carazzi's hematoxylin. Magnification: ocular 7× objective 20×.

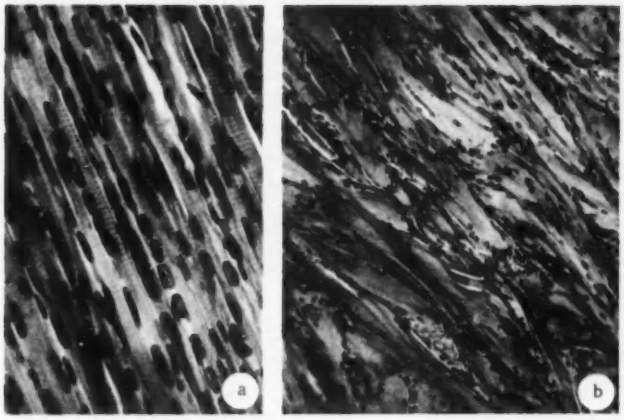

Fig. 3. Regeneration of muscle tissue 25 days after operation. Experiment using vitamin B_{12}. a) Proximal part of the regenerating tissue; the arrangement of the young muscle fibers is less chaotic than in the controls; b) regenerative reconstruction of the muscle fibers in the distal parts of the muscle (multiplication of nuclei, cleavage of myosymplasts). Stained with Carazzi's hematoxylin. Magnification: ocular 7×, objective 40×.

portions of the muscle were in a highly "excited" state: the cytoplasm of the "old" (uninjured) muscle fibers was basophilic; the number of nuclei was increased, and they occupied the central, axial part of the fiber or were arranged in a chain beneath the sarcolemma.

At later stages (15th-20th day), these distinctive features of regeneration which have been described were preserved in both the proximal and distal parts of the wound, in the newly formed muscle cells, longer than in the controls. On the 20th day, the regenerated muscle tissue consisted mainly of differentiated muscle fibers. In the distal part, however, the regenerative reconstruction of the muscles had not come to an end. On the 25th day after operation, in this zone it was possible to see extensive areas of newly formed muscle tubes and young fibers containing an abundance of nuclei (Fig. 3 a). The center of the defect, just as in the control animals, was filled with fibrous tissue, interspersed with adipose tissue. In the proximal part of the regenerating tissue the arrangement of the young muscle fibers was less chaotic than in the control animals (Fig. 3 b).

The large defect in the muscle in these rats was thus replaced by regenerating muscle and connective tissue. By comparison with the controls, in the experimental animals the reactive reconstruction of the muscle fibers was more pronounced, especially in the lateral and distal portions of the muscle. The high "densitivity" of the latter during administration of vitamin B_{12} may be due to the greater functional loading of these portions of the muscle following excision of its central area. Another important factor was presumably the partial denervation of the lateral and distal portions of the muscle as a result of injury. The preliminary results of a study of the restoration of the nerve connections in the region of the regenerating muscle showed that it was only on the tenth day that young nerve fibers grew into the region of the defect and reached its distal end.

As a result of the active regenerative reconstruction of the muscle fibers in the rats receiving vitamin B_{12}, a more intensive formation of new muscle cells was observed in these animals. The changes observed in all the experimental animals receiving vitamin B_{12}, either alone or in conjunction with thyroidin, were uniform in type and coincided with the individual stages of the process of repair. It can be assumed that during the combined action of these drugs, that of vitamin B_{12} was predominant. Evidence in favor of this is given, in particular, by the fact that in the rats receiving vitamin B_{12} and thyroidin large numbers of fat cells were deposited in the region of the wound, and there was active proliferation of connective tissue; this was not at all typical of the regenerating tissue which developed in purely hyperthyroid animals [5]. The inhibitory action of the thyroid gland hormone on the collagen-forming function of the fibroblasts was noted by V. G. Eliseev [3] and Moltke [13] in their investigations. According to the findings of Asboe-Hansen [10], this depression of collagen formation is explained by the fact that thyroxin causes a marked decrease in the content of mucopolysaccharides in the connective tissue.

SUMMARY

The author studied the effect of vitamin B_{12} on the reparative regeneration of skeletal muscle tissue in sexually mature male rats. A standard injury was inflicted on all the animals. In comparison with control animals reactive changes in the injured muscle were more pronounced in rats which received vitamin B_{12}. These changes involved increased disintegration of one part of the muscle fibers situated at some distance from the wound, as well as progressive morpho-physiological reconstruction of a large number of muscle fibers with abundant formation of myosymplasts. In rats which received vitamin B_{12} the formation of young muscular elements was intensified owing to the active regenerative reconstruction of muscle fibers.

LITERATURE CITED

1. S. V. Andreev and A. A. Znachkova, Abstracts of Proceedings of the Fourth All-Union Conference on Vitamins [in Russian] (Moscow, 1957), p. 63
2. K. M. Vintsentini and M. P. Girshman, Abstracts of Proceedings of the Fourth All-Union Conference on Vitamins [in Russian] (Moscow, 1957), p. 72
3. V. G. Eliseev and A. S. Vishniovskaya, Trudy Omskogo Med. Inst. No. 12, 121 (1948).
4. A. A. Znachkova, Doklady Akad. Nauk SSSR 109, 879 (1956).
5. T. M. Kovalenko, Arkh. Anat., Gistol. i Embriol. 34, 22 (1957).
6. F. A. Poemnyi and Sh. S. Roizen, Zhur. Nevropatol. i. Psikhiat. 57, 191 (1957).
7. N. N. Priorov and T. I. Cherkasova, Abstracts of Proceedings of the Fourth All-Union Conference on Vitamins [in Russian] (Moscow, 1957) p. 100.
8. N. N. Priorov, S. V. Andreev, and T. I. Cherkasova, Doklady Akad. Nauk SSSR 122, 312 (1958).
9. M. A. Farber, Sovetsk. Med. No. 6, 64 (1958).
10. G. Asboe-Hansen, Acta dermat-venereol. 30, 221 (1950).
11. L. W. Charkey, H. S. Wilgus, A. R. Patton, et al., Proc. Soc. Exper. Biol. and Med. 73, 21 (1950).
12. D. Leroy and I. Robin, Semaine hop. Paris 31, 93 (19/2) (1955).
13. E. Moltke, Proc. Soc. Exper. Biol. and Med. 88, 596 (1955).

DESQUAMATION OF THE EPITHELIUM OF THE VILLI OF THE SMALL INTESTINE—AN ELEMENT OF FUNCTIONAL MORPHOLOGY OR AN ARTEFACT ?

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It has been shown by many research workers [5, 7, 8, 9, 12, 15, 16, 19] that the solid part of the intestinal juice consists mainly of desquamated and disintegrating intestinal epithelial cells, i.e., that the secretion of the solid part of the intestinal juice is connected with the destructive phase of physiological regeneration [2, 10]. Different views exist, however, of the mechanism of death of the intestinal epithelial cells.

After fixation of the intestine in a stretched condition [14], a picture of the dislodgement of cells from the apices of the villi was observed. In some cases this process affected a single cell, and in others, groups of cells, but the breakdown in the integrity of the epithelial layer was always connected with destructive changes in the epithelial cells, and with their necrosis. Similar findings were obtained by other authors [11, 13, 17].

M. I. Razumov [6] considers that the epithelium is desquamated by means of the detachment of large sheets of cells, affecting not only the apices of the villi, but also their lateral surfaces, so that their stroma is denuded. Histological and histochemical investigation showed that the desquamating epithelium did not differ from that left behind on the villi. The process of desquamation of the epithelium is preceded by the formation of epithelial tubes, the lumen of which was described earlier as Gruenhagen's spaces. Other authors have reported similar findings [3, 4].

In view of the divergent opinions on this subject, we carried out the present investigation, based on the findings of those authors [18, 20] who consider that the formation of Gruenhagen's spaces is the result of the contraction of the muscle fibers of the stroma of the villi due to the fixing agent and the ensuing isolation from the reticular stroma of the villi of the thickened epithelium, loosely connected to it.

We believe that the detachment of the epithelial layer from the stroma is brought about by the adhesion of the villi to each other or to the undigested food mass, since the apex of the villus is attached under these circumstances. Desquamation and detachment of the epithelium takes place during fixation and embedding to a greater degree the closer the contact between the villi.

In order to test this hypothesis, the intestines of 48 rats were irrigated in situ, with slight distension, at first with physiological saline for 2 min, and later with Bouin's fluid for 5 min. The lumen of the intestine was moderately distended. Histological examination of fragments taken from four different sections of the small intestine showed that, in certain villi, detachment of the epithelium from the stroma took place, although in none of the sections of the small intestine in all the rats investigated were villi with a completely denuded stroma found. With distension of the lumen of the intestine, and consequent separation of the villi from each other, in a modification of the experiment, similar results were obtained. Small areas of different sections of the intestine (in 3 dogs and 5 rats) were ligated on two sides with silk thread, and physiological saline or fixing agent was injected into the lumen of the bowel until the muscular wall was distended. As controls, neighboring areas of the intestine were used, which were not ligated nor filled with fluid. Villi deprived of their epithelium were never visible in the distended areas (Fig. 1). In the control areas a different picture was often visible, with disturbance of the integrity of the epithelial layer, as has been described in detail by other authors [3, 4, 6].

This group of experiments also includes the fixation of fragments of intestine (of 50 rats, 30 guinea pigs, and 3 Macacus rhesus monkeys), followed by cutting into segments and placing over a strip of cardboard without stretching. As a result of the contraction of the muscularis mucosae, the mucous membrane was everted. By the use of various fixatives (Bouin's fluid, 10% formalin solution, Carnoy's mixture) we never once observed villi with a denuded stroma.

The prevention of contact between the apices of the villi thus prevented the separation of the epithelial layer

Fig. 1. Intestinal villi of a dog, the walls of the intestine being stretched with physiological saline. The epithelial layer is in close contact with the stroma and plicae are visible on the lateral surfaces. Magnification 7·20. Stained with hematoxylin-eosin.

from the stroma of the villi and the denudation of the stroma as a result of fixation. We consider that, because of this, two hours after rats have been taken food and the intestine is distended by the juices of the digestive glands, the patterns of detachment of the epithelial layer and denudation of the stroma of the villi is not observed in histological preparations [1].

Prevention of the contraction of the muscle fibers of the villi at the moment of fixation must lead [20] to the obtaining of normal patterns of structure of the intestine. This may be achieved in various ways. We cooled pieces of intestine (from 5 rats)for periods of from 10 minutes to 2 hours at +4° and fixed them in formalin at room temperature. On histological examination, the structure of the villi was found to be normal, and they were elongated in shape. The same result was observed after fixation of pieces of intestine (5 rats and one dog), at a temperature close to that of the body, in formalin cooled to +4°.

In the intestine of rabbits, Z. M. Gadzhieva [4] did not observe the desquamation of large sheets of epithelial cells, and she considers that there are no muscle fibers in the stroma of the villi in rabbits. It seems to us that this would also explain the absence of formation of obvious epithelial tubes.

On the assumption that fixation of the intestine at a moment when the muscle fibers were contracted might disclose the pattern of the relationship between the epithelial layer and the stroma, we perfused the intestine (of 5 rats) with warm physiological saline at a pressure of 30-35 mm Hg for 5 min, and with Bouin's fluid for 10 min. It will be seen from the photomicrograph (Fig. 2) that the epithelial layer is in close contact with the stroma; the muscle fibers are round in shape, i.e., they are contracted. After fixation of the intestine in Bouin's fluid, immediately after sacrifice of rats (5 animals) kept on the same diet, the typical pictures of desquamation of the epithelium from the stroma of the villi were seen (Fig. 3), which were described by Z. M.

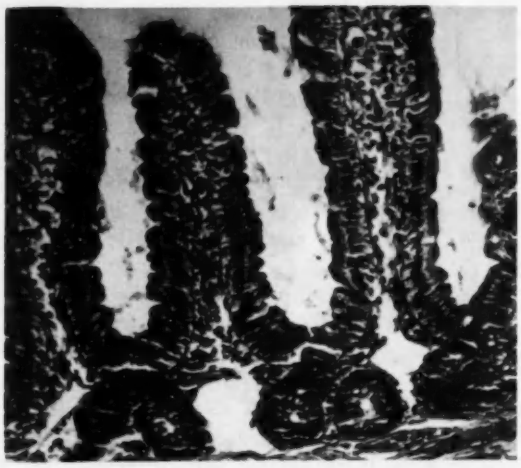

Fig. 2. Villi of a rat's intestine. Irrigation with physiological saline and fixation. No detachment of the epithelium from the stroma. Plicae of epithelium are seen. Muscle fibers round in shape. Magnification 7·40 Stained with hematoxylin-eosin.

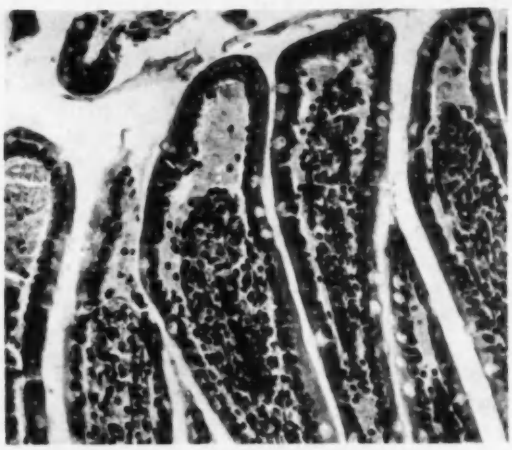

Fig. 3. Villi of a rat's intestine, fixed immediately after sacrifice. Detachment of the epithelium from the stroma is seen. Magnification 7·40. Stained with hematoxylin-eosin.

Gadzhieva [3, 4] as "elements of the functional morphology of the small intestine". Irrigation of the intestine in dogs for the same period of time did not completely abolish the phenomenon of detachment of the epithelium from the stroma. This may evidently be due to the more powerful musculature and to the well-developed Kerkring's valves, for in places where these valves were only feebly developed, the villi were completely covered by epithelium.

In order to verify the fact that villi with denuded stroma were not present in the normal intestine, the intestine of 5 rats were perfused with physiological saline for 5-10 min. The intestine was then separated from the mesentery, incised longitudinally, placed over cardboard with the mucous membrane outermost, and fixed in Bouin's fluid. Examination with the stereoscopic microscope (MBS-1) failed to show a single villus without epithelium although the epithelial layer could readily be separated and detached at the apices of the villi by means of a dissecting needle.

The same picture was observed during the examination of the mucous membrane of different portions of the intestine (in 3 Macacus rhesus monkeys) immediately after sacrifice, by means of the stereoscopic microscope.

Z. M. Gadzhieva [3] carried out histological investigations of the intestine of rats without preliminary irrigation, and found detachment of the epithelium and denudation of the stroma in certain areas of the small intestine. If this was not an artefact of fixation, we undoubtedly should be able to find desquamation of the epithelial layers in our experimental rats, even though only in a few places.

We performed experiments on five rats, in which we examined the intestine under the stereoscopic microscope during life. For this purpose, after starving the rats for 48 hours, we opened the abdomen under ether anesthesia; a small area from the proximal division of the jejunum was excised at the opposite border to the attachment of the mesentery, and its edge was sutured to the skin. The mesentery of this area of intestine was left behind in the abdomen. The mucous membrane was irrigated with physiological saline and kept in a moist and warm condition throughout the period of observation. For six hours we found no essential changes in the external appearance of the villi, and their blood supply was well maintained. No signs of detachment of the epithelium were present.

Similar experiments were conducted on three rats which had taken food, the observations lasting four hr, and in no case did we find any changes in the epithelial layer of the villi.

When the data of M. I. Razumov and Z. M. Gadzhieva is analyzed, it is easy to find a number of contradictions in their conclusions. They associate the phenomenon of desquamation of the epithelial cells with the secretion of the solid part of the intestinal juice. In their view, living, unchanged cells are detached, whereas in the juice secreted periodically there are found cells undergoing destruction, with pyknosis or karyorrhexis of their nuclei.

The epithelial plicae on the lateral surfaces of the villi were interpreted by M. I. Razumov as an accumulation of epithelial cells, preceding the formation of epithelial tubes. Other authors [20] considered that these plicae are the result of the contraction of muscle fibers, leading to a reduction of the volume of the stroma. The same state of affairs could be seen in our own preparations, in which irrigation of the intestine with warm physiological saline caused contraction of the muscle fibers of the stroma (Fig. 2).

For the proof of a number of his postulates, M. I. Razumov had to admit that renewal of the epithelial cells takes place as a result of their formation from the reticular stroma. This assumption is contradicted by numerous morphological findings, collected in the course of the last decades.

On the basis of data in the literature and of the results of our experiments, we conclude that the phenomena described in the papers by M. I. Razumov and Z. M. Gadzhieva must be regarded not as "elements of the functional morphology of the small intestine," but rather as artefacts arising as a result of autolysis, fixation and subsequent histological treatment of areas of the intestine without taking into consideration the possible disturbance of structural relationships. It hence follows that, during the analysis of the phenomenon of detachment of the intestinal epithelium, i.e., of the destructive phase of its physiological regeneration, and of certain of its characteristic pathohistological patterns, the possibility of the production of artefacts must be taken into consideration.*

SUMMARY

As shown on rats, dogs, guinea pigs and monkeys, detachment of the epithelial layer from the stroma of intestinal villi with exposure of the stroma is actually an artefact. The latter may occur in autolysis, fixation and histological treatment of material. The normal interrelationship of the epithelium and the stroma may be obtained by fixation in a cooled solution or by stretching the intestine, thus preventing the villi from coming in contact with each other.

LITERATURE CITED

1. S. L. Blyakher, Abstracts of Proceedings of a Conference of Junior Scientific Workers of the Intitute of Pharmacology, Experimental Chemotherapy and Chemoprophylaxis [in Russian] (Moscow, 1953) p. 5.
2. M. A. Vorontsova and L. D. Liozner, Physiological Regeneration [in Russian] (Moscow, 1955).

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3. Z. M. Gadzhieva, Byull. Éksp. Biol. i Med. No. 11, 100 (1957).†
4. Z. M. Gadzhieva, Voprosy Pitaniya, No. 6, 65 (1957).
5. A. Dobroslavin, Voen—Med. Zhur. Part. 107, Section 2, 80 (1870).
6. M. I. Razumov, Voprosy Pitaniya No. 4, 18 (1952).
7. N. D. Strazhesko, Physiology of the Intestines (Dissertation) [in Russian] (St. Petersburg, 1904).
8. L. S. Fomina, Secretion of Intestinal Enzymes (Doctoral dissertation) [in Russian] (Moscow, 1956).
9. N. P. Shepoval'nikov, Physiology of the Intestinal Juice (Dissertation) [in Russian] (St. Petersburg, 1899)
10. G. K. Shlygin, Uspekhi Sovremennoi Biol. 32, 14 (1952).
11. R. B. Fisher and D. S. Parsons, J. Physiol. 110, 36 (1949).
12. K. W. Florey, R. D. Wright, and M. A. Jennings, Physiol. Rev. 21, 36 (1941).
13. C. S. Hooper, and M. Blair, Exper. Cell. Res. 14, 175 (1958).
14. C. P. Leblond and C. E. Stevens, Anat. Rev. 100, 357 (1958).
15. W. Leube, Zbl. med. Wiss. No. 19, 289 (1868).
16. R. M. H. McMinn, J. Anat. 88, 527 (1959).
17. L. Mendel, Arch. ges. Physiol. 63, 425 (1896).
18. W. Patzelt, W. Möllendorf Handbuch der mikroskopischen Anatomie der Menschen 5 (Berlin, 1936), pp. 1-404.
19. F. Röohman, Arch. ges. Physiol. 41, 411 (1887).
20. G. Wolff-Heidegger, Z. Wiss. Mikr. 56, 417 (1940).

† Original Russian pagination. See C. B. translation.

THE EFFECT OF DIVISION OF THE ANTERIOR AND POSTERIOR ROOTS OF THE SPINAL CORD ON THE MITOTIC ACTIVITY OF THE EPIDERMIS AND THE RATE OF HEALING OF CUTANEOUS WOUNDS IN RATS

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We have previously shown [5, 6] that unilateral division of the nerves of the hindlimb (femoral, obturator and sciatic) leads to a decrease in the mitotic activity of the epidermal cells and to a slowing of the healing of cutaneous wounds in the denervated limb.

Since the nerves divided were mixed, it was impossible to discover on account of which fibers—afferent or efferent— it was that their exclusion led to these changes.

The existing data [1, 3, 9] is limited and contradictory, and does not permit any definite conclusions to be drawn about the influence of the afferent and efferent innervation on the mitotic activity of the tissues and the rate of wound healing. Meanwhile, the investigation of these problems is of great importance to the elucidation of the laws governing the regulation of regenerative processes and cell division.

In the present work we studied the effect of either purely afferent or purely efferent nerves on cellular proliferation and on the rate of healing of skin wound.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male white rats (average weight 150 g). Depending on the character of the operation, the rats were divided into three groups, each of seven animals. In the animals of the first group, the anterior roots of the spinal cord were divided on the left side, the posterior roots being preserved; in animals of the second group both anterior roots were divided; finally, in the animals of the third group, the posterior roots were divided and the anterior roots left intact.

Division of the roots was carried out under ether anesthesia, by the method suggested by G. N. Kryzhanoskii. In all cases the last thoracic, all the lumbar and the first sacral roots were divided . In the postoperative period tests of the completeness of the denervation were performed on two occasions by means of the application of painful stimuli to the denervated and intact limbs.

On the tenth day after operation a piece of skin for counting the number of mitoses was excised from the lateral aspect of the upper third of both legs in all the animals. The pieces of skin measured 4 · 8 mm. The wounds thus formed were used for the subsequent study of the rate of wound healing. Simultaneously with the infliction of skin wounds to the hindlimbs of animals on which the operation of division of the anterior or posterior roots had been carried out, wounds of the same size were also inflicted to the forelimbs in the region of the forearm. The material for counting mitoses was fixed in Bouin's fluid and embedded in celloidin-paraffin wax. Sections 7μ in thickness were stained with hematoxylin by Carazzi's method. The mitotic activity and the rate of healing of cutaneous wounds were estimated by the methods described in our previous papers [4, 5]. The observations were continued for a period of 40 days after denervation.

TABLE 1. Mitotic Coefficient in the Epidermal Cells of the Skin of Rats after Division of the Anterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)		Difference between mitotic coefficients of limbs as a percentage of intact limb
in the intact limb	in the denervated limb	
10,3	7,3	70,87
9,9	5,4	54,54
7,9	2,3	29,12
7,3	6,2	84,93
6,3	3,8	60,31
5,8	3,8	65,51
5,2	5,6	107,69
Mean 7,6	4,9	64,5

TABLE 2. Mitotic Coefficient in the Epidermal Cells of the Skin in Rats after Division of the Anterior and Posterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)		Difference between mitotic coefficients of limbs as a percentage of intact limb
in the intact limb	in the denervated limb	
6,5	4,7	72,30
6,4	1,6	25,00
5,8	1,7	30,00
5,7	2,6	45,61
5,1	2,5	49,01
5,0	4,2	84,00
3,0	1,9	63,33
Mean 5,4	2,7	50,00

EXPERIMENTAL RESULTS

Figures showing the mitotic activity in rats after division of the anterior roots of the spinal cord are given in Table 1, from which it is clear that the mitotic coefficient in the epidermis of the denervated limbs is lower in nearly every case than in the epidermis of the intact limbs.

The difference between the mean mitotic coefficients was significant (P= 0.01). Identical results were obtained after the simultaneous division of both anterior and posterior roots (Table 2). In this case too, the difference between the mean values was significant (P= 0.001).

Counts of the number of mitoses after division of the posterior roots showed that the mean mitotic coefficient in the two limbs was almost the same: In the limbs with afferent nerves intact it was 4.1 ‰, and in the limbs with afferent nerves destroyed it was 4.2 ‰ (Table 3).

It must be pointed out, however, that in the rats in which the anterior roots were divided , the mitotic activity in the epidermis of both limbs was considerably higher than in the animals in which both anterior and posterior roots had been simultaneously divided. This difference is close to significant: Its value in the denervated limbs was P = 0.02, and in the intact limbs P = 0.03.

The number of the different phases of mitosis in the epidermis of the limbs with disturbed innervation showed no essential difference from their number in the epidermis of the intact limbs. This showed that the division of the anterior or posterior roots of the spinal cord, just as the simultaneous division of both, does not alter the rate of the course of any of the phases of mitosis. It may thus be considered that the decrease which was found in the total number of mitoses in the epidermis of the denervated limbs was dependent on the smaller number of cells taking part in division and not on any increase in the duration of the process of cell division itself, at any of its phases.

The study of the process of healing of cutaneous wounds in the rats with division of the anterior roots of the spinal cord showed that the wounds of both fore- and hindlimbs healed in roughly the same periods of time (17-20 days).

In rats in which the anterior and posterior roots had been simultaneously divided, wounds were inflicted on the hindlimbs alone. In this group of animals, healing of the wounds of the intact limbs took place without any apparent abnormality and was complete on the 10th-12th day. Soon after the infliction of wounds on the thigh and leg of the denervated limbs in two rats, ulcers developed, which showed rapid progression. Gangrene of the limb ultimately developed, and this was probably the cause of their comparatively rapid death (on the 6th-8th day). In one rat a large ulcer formed at the site of the wound, and in two other rats the wounds neither healed nor increased in size. In the remaining two animals the wounds healed on the 18th-20th day.

The simultaneous division of the anterior and posterior roots thus greatly complicated the process of wound healing or led to the formation of trophic ulcers.

In animals in which the posterior roots of the spinal cord had been divided, the wounds on the forelimbs and on the hindlimbs with their intact afferent nerve supply healed at approximately the same rate (10-12 days).

TABLE 3. Mitotic Coefficient in the Epidermal Cells of the Skin in Rats after Division of the Posterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)	
in the intact limb	in the denervated limb
8,1	2,2
7,3	10,0
6,1	5,1
2,8	3,1
1,9	6,6
1,6	0,9
1,2	0,3
Mean 4,1	4,2

On the limbs deprived of their afferent nerve supply, in all the rats except one, ulcers developed at the site of the wounds. It must be pointed out that, in these same rats, ulcers were also formed at the back of the foot of the limbs on the site of the posterior root section, at different intervals of time after the operation (on the 19th-32nd day), in those areas which were most exposed to trauma during the animal's movements.

Under experimental conditions, the formation of trophic ulcers on the foot was thus observed only in those animals in which the posterior roots had been divided, which demonstrates the important role of the afferent innervation in trophic disorders of the tissues. Our findings were in agreement with the views of T. A. Grigor'eva [2], who considers that trophic disorders arise in the tissues as the result of the disturbance of the function of the peripheral afferent neurone, and that these processes are directly dependent on the degree of destruction of the afferent nerve supply. I. D. Khlopina [7, 8] maintains another point of view namely that the disturbance of the trophism of the tissues does not depend on the exclusion of a particular component of the nervous system, but on the degree of denervation. According to her findings, the development of trophic ulcers is observed only in those cases when the roots are divided distally to the spinal ganglion.

In our experiments, the posterior roots were divided proximally to the ganglia, yet nevertheless ulcers were formed in six of the seven rats. The principal condition which could lead to the development of ulcers of the foot, in our opinion, was the additional irritation which resulted from the excision of pieces of skin for counting of the mitoses. This hypothesis is in full agreement with the views of A. D. Speranskii [6] concerning the "second blow".

The development of trophic ulcers in animals after section of the posterior roots and after the simultaneous section of the anterior and posterior roots, and the absence of ulcers after section of the anterior roots alone, is evidence that this process is not dependent on the degree of denervation, but on which roots are divided.

SUMMARY

Unilateral section of the anterior roots of the spinal cord in rats leads to the reduction of mitotic activity of the epidermal cells in the limb thus deprived of its afferent nerve supply and has no significant effect on the rate of wound healing. Section of posterior roots does not produce any change in the number of dividing cells in the epidermis of the extremities but results in the development of trophic ulcers. Simultaneous division of both anterior and posterior roots of the spinal cord leads to reduction of mitotic activity of the epidermal cells in the operated extremity; in a number of cases it provokes the formation of trophic ulcers at the site of the wound infliction or the development of gangrene.

Our results thus demonstrate that the exclusion of a particular form of innervation differs in its effect on the mitotic activity of the epidermal cells and on the rate of healing of cutaneous wounds.

LITERATURE CITED

1. V. G. Alekseeva and F. K. Levina, Problems of the Functional Correlation of the Vegetative Nervous System. B. [in Russian] (Moscow, 1937), pp. 92-112.
2. T. A. Grigor'eva, Doklady Akad. Nauk SSSR 78, 387-390 (1951).
3. M. K. Zakharov, Arkh. Anat. Gistol. i Embriol. 32, 47-53 (1955).
4. A. N. Kulagin, Byull. Éksp. Biol. i Med. No. 1, 97-100 (1959).*
5. A. N. Kulagin, Problems of Regeneration and Cell Division [in Russian] (Moscow, 1959), pp. 266-270.
6. A. D. Speranskii, Elements in the Construction of the Theory of Medicine [in Russian] (Moscow, 1937).
7. I. D. Khlopina, Doklady Akad. Nauk SSSR 86, 205-208 (1953).
8. I. D. Khlopina, Morphological Changes in Denervated Tissues and the Development of a Trophic Ulcer of Neurogenic Origin [in Russian] (Leningrad, 1957).
9. S. Sigelman and J. S. Friedenwald, Arch. Ophth. 52, 46-57 (1954).

* Original Russian pagination. See C.B. Translation.

THE DEVELOPMENT OF SYNESTROL-INDUCED CHANGES IN THE REPRODUCTIVE SYSTEM OF THE MALE RABBIT

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Considerable changes are brought about in the male reproductive system by the action of estrogens. Several investigations have been devoted to their experimental study. In particular, changes in the accessory sex glands of the rabbit were described in our previous paper [1], in which the relevant literature is cited.

The question of the permanence of the estrogen-induced changes, however, has received little study. There are observations [3] of the restoration of the normal relationships in the genitourinary system of mice after cessation of the administration of polyanol.

During the study of the changes in the testes in mice under the influence of synestrol and testosterone propionate, the partial or total regression of these changes was observed [5] after discontinuation of the hormones.

In the present research we investigated the development of changes due to synestrol in the reproductive system of male rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 28 male rabbits, weighing on the average 2.0-2.5 kg, which received regular subcutaneous injections thrice weekly of 0.45 ml of a 2% oily solution of synestrol (0.009 g synestrol). Eleven rabbits died from intercurrent diseases (pasteurellosis, pneumonia) during the first three months (i.e., during the administration of synestrol), and these serve as controls. The remaining 17 rabbits received synestrol injections for three months (a total of 0.351 g of the pure substance). The experiment lasted for 30 to 464 days after cessation of the administration of synestrol. The material was fixed in 10% formalin and embedded in celloidin-paraffin wax. Histological sections were stained with hematoxylin-eosin and by Van Gieson's method. Individual sections were stained with mucicarmine and toluidine blue, and by Gram's, Mallory's and Heidenhain's methods.

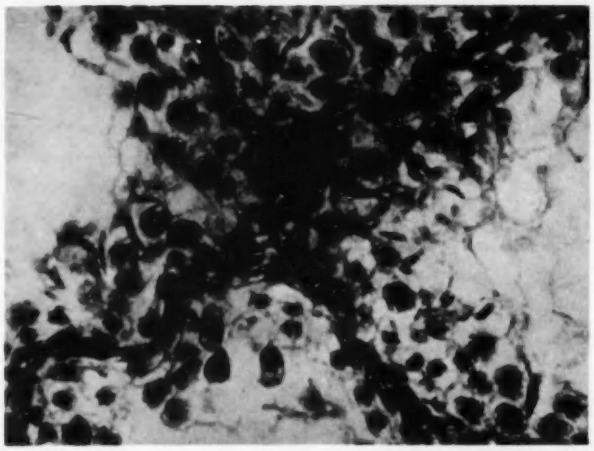
EXPERIMENTAL RESULTS

The administration of synestrol was accompanied by the development of a complex group of changes in the accessory sex glands. At an early stage, hyperemia and edema developed and the stroma and epithelium were infiltrated with pseudoeosinophilic leukocytes and lymphocytes. Signs of degeneration were observed in the epithelium: karyorrhexis, desquamation, and more rarely, vacuolation of the cells. Secretion was diminished and ceased altogether, and the epithelium gradually lost its organ-specific differentiation and underwent atrophy. At the same time these changes were accompanied by focal proliferation of the epithelium and its metaplasia into transitional and stratified squamous epithelia, with keratinization in certain cases.

In the urethra, at the level of the prostatic vesicle and distally to it, infiltration of the stroma and epithelium with pseudoeosinophilic leukocytes and lymphocytes developed under the influence of synestrol. The accumulation of leukocytes and cellular detritus caused a cystic dilatation of the glandular crypts. In the epithelium, proliferation was observed along with the loosening and destruction of the individual cells. In some cases the transitional and prismatic epithelium in the distal part of the urethra was replaced by stratified squamous epithelium.

In the testes, spermatogenesis was arrested under the influence of the synestrol. The cells of the spermatogenic epithelium and also a proportion of the Sertoli cells degenerated. In the majority of the convoluted tubules, therefore, only one row of epithelial cells was preserved, lying on the basal membrane. Under these circumstances the convoluted tubules decreased sharply in size as they descended. Occasional tubules were hypertrophied. An increase in the number of interstitial cells was observed in the stroma.

After the administration of synestrol had been discontinued, a rapid disappearance of the infiltration with lymphocytes and leukocytes in the accessory sex glands was observed. On the 30th day no infiltration could be seen in the majority of the glands, and only in the seminal vesicle did small collections of lymphoid cells remain until 2 ½ months.



Renewal of spermatogenesis in the convoluted tubules of the testes. Mitoses. Experiment showing the action of synestrol. Duration of observations 120 days. Stained with hematoxylin-eosin. Zeiss 8× ocular, Zeiss 8 × objective magnification 600 ×.

Proliferation of the epithelium ceased at the same time. The epithelium, where previously it had undergone proliferation, now regressed. One basal layer of cells remained, and the overlying layers were desquamated. In the prostatic vesicle small groups of cells sometimes disintegrated in situ, to form intraepithelial cysts. Their nuclei were dissolved and their protoplasm was converted into an amorphous mass, giving a mucin reaction. Small areas of epithelial proliferation, without metaplastic differentiation into stratified squamous epithelium, underwent regression during the period of 1-2½ months after the administration of synestrol had been discontinued. The layers of stratified squamous epithelium degenerated more slowly. In the prostatic vesicle they were present until the sixth month.

The keratinized masses in the lumen of the glandular alveoli were absorbed with the aid of leukocytes the number of which decreased with time. The leukocytes were destroyed and their debris was absorbed by macrophages, which were converted into foam cells. The residues of the keratinized masses swelled, turning into a homogeneous, colloidal mass, which was gradually evacuated through the ducts. Small remnants of stratified, keratinized masses, which had lost their property of staining by Gram's method, were encountered in the lumen of the alveoli of the glands, however, at much later periods of the experiment (212, 326 and 464 days).

In the immediately following months, as regression of the sunestrol-induced changes in the accessory sex glands proceeded, signs of atrophy became predominant. The changes in the accessory sex glands at this period were identical with those observed after castration.

One month after discontinuation of the administration of synestrol, no infiltration with lymphocytes and leukocytes was observed in the urethra. Detritus from the dilated glandular diverticula had been evacuated and the diverticula were smaller in size. After 2½ months, at the level of the prostate gland and distally to it, the transitional epithelium was thinned out and, in places was replaced by a two-layered prismatic epithelium, which was contantly found in this region at later periods. Until 5½ months, a stratified epithelium resembling stratified squamous epithelium was sometimes found in the distal portion of the urethra.

In the testes, the collapse and atrophy of the convoluted tubules, caused by synestrol, could be maintained until six months. Individual epithelial cells continued to degenerate. In other cells, however, occasional mitoses appeared at the end of the second month. In some animals, after four months, proliferation of the spermatogonia could be observed in the majority of the convoluted tubules. In other animals, proliferation was observed later, although eight months after administration of the hormone had been discontinued, a well-merked spermatogenesis was observed in all the animals (see Figure). The diameter of the convoluted tubules had increased to normal and the number of interstitial cells had diminished.

Restoration of the normal structure and function of the testes took place parallel with the restoration of the structure and function of the accessory sex glands. The first signs of restoration of the lost organ-specific differentiation appeared at the end of the fourth month. The glandular crypts in the seminal vesicle then became

deeper, and the epithelial cells increased in height. Proliferation of the epithelium was observed and, in connection with the accumulation of secretion, the vesicle became dilated. In the prostatic vesicle, at the fourth month, the majority of the alveoli were still in a collapsed state, but in places the epithelium had become higher, and the protoplasm, which had increased in amount, had become oxyphilic, and the oval nucleus was situated in the middle part or the lower half of the cell. At later periods the dimensions of the alveoli of the glands increased. Mitoses were encountered in the epithelial cells. Differentiation of the epithelium from a focal type became universal. Secretory granules appeared in the protoplasm of the cells and the lumen of the alveoli. The plication of the glandular alveoli increased sharply. At the end of one year the gland had acquired its normal appearance. The collapse and atrophy lasted slightly longer in the prostate gland than in the prostatic vesicle. An obvious increase in the height of the epithelial cells and a characteristic basal arrangement of the nuclei could be observed after seven months. Concurrently, dilatation of the glandular alveoli, mitoses in the epithelial cells and the formation of granules of secretion were noted. At the end of one year the gland had returned to its normal state, and many corpora amylacea were formed in the lumen of the alveoli.

The paraprostatic and bulbo-urethral glands underwent relatively slight changes during atrophy. On their return to the normal state, they showed a slight increase in the dimensions of their epithelial cells and restoration of the lumen of their terminal divisions.

The changes induced by synestrol in the accessory glands of the male reproductive system in the rabbit were thus temporary and gave way to regression. Two periods could be distinguished in the course of this regression: a period of disappearance of the infiltrative and proliferative manifestations, lasting less than one month, and a period of atrophy, lasting up to one year, and replaced by the gradual restoration of the normal, typical structure of these organs.

The morphological changes are evidence of the great metabolic disturbances produced by the action of synestrol on the male reproductive system. The hyperemia, leukocytic infiltration and epithelial proliferation taking place in these conditions suggest that synestrol increases the intensity of metabolism in the accessory sex glands, at least during the first months. The depression of the function of the testes, however, indicates the opposite action.

The complex and divergent character of the action of synestrol may account for the temporary nature of the proliferative manifestations which it induces in the accessory male sex glands. It must be considered that the "chemical castration" caused by the estrogen in the male depresses proliferation and metaplasia of the epithelium when the administration of the hormone is discontinued.

This view is in agreement with reports in the literature [2, 6] that carcinoma of the reproductive system cannot be produced in males by the action of synestrol, whereas it does occur in females. An exception to this is the communication of V. P. Konoplev [4].

SUMMARY

Synestrol was administered to 28 rabbits for a period of 3 months (a total of 0.351 gm).

After discontinuance of its administration—the experiments lasting from 30 to 464 days—a regression of the changes in the accessory sex glands was noted, the course of which could be subdivided into two periods, viz., the period of liquidation of the infiltrative proliferative phenomena (lasting less than a month), and the period of atrophy (lasting up to one year and depending on the atrophy of the testes). The typical normal structures of the organs are restored at the end of the year.

LITERATURE CITED

1. A. G. Bobkov, Byull. Éksp. Biol. i Med. 48, 101 (1959).*
2. W. Gardner, Advances in the Study of Cancer 1 [Russian translation] (Moscow, 1955), p. 122.
3. B. V. Klyucharev, Arkh. Patol. 10, 44 (1948).
4. V. P. Konoplev, Collection: Problems of Oncology. Transactions of the AMN SSSR No. 7, 20 (1954) [in Russian].
5. V. V. Chikov, Changes Developing in the Testes under the Influence of Testosterone Propionate and Synestrol (Dissertation) [in Russian] (Leningrad, 1956).
6. H. Burrows, Biological Actions of Sex Hormones (The University Press, Cambridge, 1949).
7. H. A. Levey and C. M. Szego, Amer. J. Physiol. 183, 371 (1955).

* Original Russian pagination. See C.B. Translation.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

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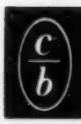

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